Interferon-α as an immunotherapeutic protein

Diana L. Brassard, Michael J. Grace, and Ronald W. Bordens

Bioanalytical Development, Schering-Plough Research Institute, Union, New Jersey

Abstract: Interferon-α (IFN-α) has proven to be a clinically effective antiviral and antineoplastic therapeutic drug for more than 16 years. During this time, evidence from in vitro laboratory studies and the clinical arena has supported the concept that IFN-α is an immunotherapeutic drug. By regulating a diverse set of cytokines and their receptors, IFN-α is uniquely positioned to prime the host immune response and provide an effective antineoplastic- and antiviral-immune response. IFN-α stimulates the innate cell-mediated response and then participates in the transition of the initial host innate response into an effective adaptive-immune response. IFN-α also drives the adaptive cell-mediated CD8+ T-cell response and helps to maintain a CD4+ Th1-cell population balance for an effective antineoplastic and antiviral host defense. This review will describe the current state of knowledge of IFN-α as an immunoregulatory protein and address specific issues of IFN-α as an immunotherapeutic for antineoplastic and antiviral diseases. *J. Leukoc. Biol.* 71: 565–581; 2002.

Key Words: antiviral therapy · antitumor therapy · immunological therapy

INTERFERON-α (IFN-α), THE QUIET IMMUNE-REGULATORY CYTOKINE

First described as a protein secreted by fibroblasts, the IFN-α cytokine was shown to induce paracrine resistance against lytic virus infection [1]. Yet, an active virus infection was not required solely for the induction of IFN-α, because expression could also be induced by the treatment of cells with endotoxins, dsRNA, poly (I:C), or CpG [2–5]. Later, an IFN-α homologue was identified from lymphoid cells, the IFN-γ protein that could also be expressed by nonlymphoid cells [6, 7]. These experiments led to the classification of IFN-α and IFN-β as Type I and IFN-γ as Type II IFNs. It now appears that IFN-α/β and IFN-γ have nonredundant and functionally complementary activities in the host response to viral infection [8–10].

A thorough analysis of IFN-α expression shows that IFN-α is secreted not only by fibroblasts but also by T cells, macrophages, plasmacytoid monocytes, dendritic cells (DCs), and natural killer (NK) cells [2–4, 11, 12]. More recently, IFN-α has been classified as the "leukocyte interferon" [13]. This designation was intended to refer to the principal host cell secreting the IFN rather than to serve as a comment on the cytokine's immunologic mechanism of action. The current working model for IFN-α proposes that the "professional" IFN-α/β secretor cells are the CD4+CD11c-type 2 DC precursors (pDC2s) [14–16]. The pDC2 cells secrete between 200 and 1000 times more IFN-α than any other white blood cell [16]. Because DCs are important to the migration of activated T cells to injured/infected tissue, IFN-α is involved indirectly in regulation of the local immune response [17]. The putative role of the pDC2 also highlights an important aspect of IFN biology: that the regulation of patient-innate and adaptive-immune responses to IFN-α therapy may vary depending on dose and administration schedule. This concept serves to emphasize the potentially important role that a low-dose, systemic, therapeutic administration of IFN-α during antineoplastic or antiviral treatment may serve mechanistically as a priming cytokine for the host immune response [15, 18].

IFN-α primes the host immune response

IFN-α and IFN-γ are important to host immune defense against neoplastic and viral diseases [8–10]. Biron [19] was perhaps the first reviewer to state clearly, "IFN-α/β play a dominant role in shaping downstream innate and adaptive immune responses to viral infection" (**Fig. 1**). Because IFN-α expression occurs as an early response to infection, it precedes a majority of the other innate-immune response cytokines. In fact, the timing of IFN-α expression after infection suggests that its primary role is to induce a priming state during the initial immune response to infection [20–22]. This IFN-α-induced priming activity is thought to augment the host primary-immune response to viral infection [18]. However, IFN-α activity on the immune system also shows an overlapping function (and in some instances, a synergy) with other "early response" cytokines [e.g., transforming growth factor-α (TGF-α) and interleukin (IL)-2; 23]. Thus, it has been suggested that IFN-α is the first and most important cytokine secreted by antigen-presenting cells (APC) after antigen stimulation by a T-helper cell type (Th)0 cell [21]. The phenomena known as priming was first shown when it was observed that a low-dose IFN-α treatment preceding viral, endotoxin, or poly(I:C) challenge resulted in an increased protection from viral challenge [3, 18]. An IFN-α-induced priming response is also seen with IL-2 production in mitogen-activation models that are enhanced by IFN-α pretreatment [23].

Correspondence: Michael J. Grace, Bioanalytical Development, Schering-Plough Research Institute, U-13-2-2050, 1011 Morris Ave., Union, NJ 07083. E-mail: michael.grace@spcorp.com

Received October 1, 2001; revised December 18, 2001; accepted December 20, 2001.

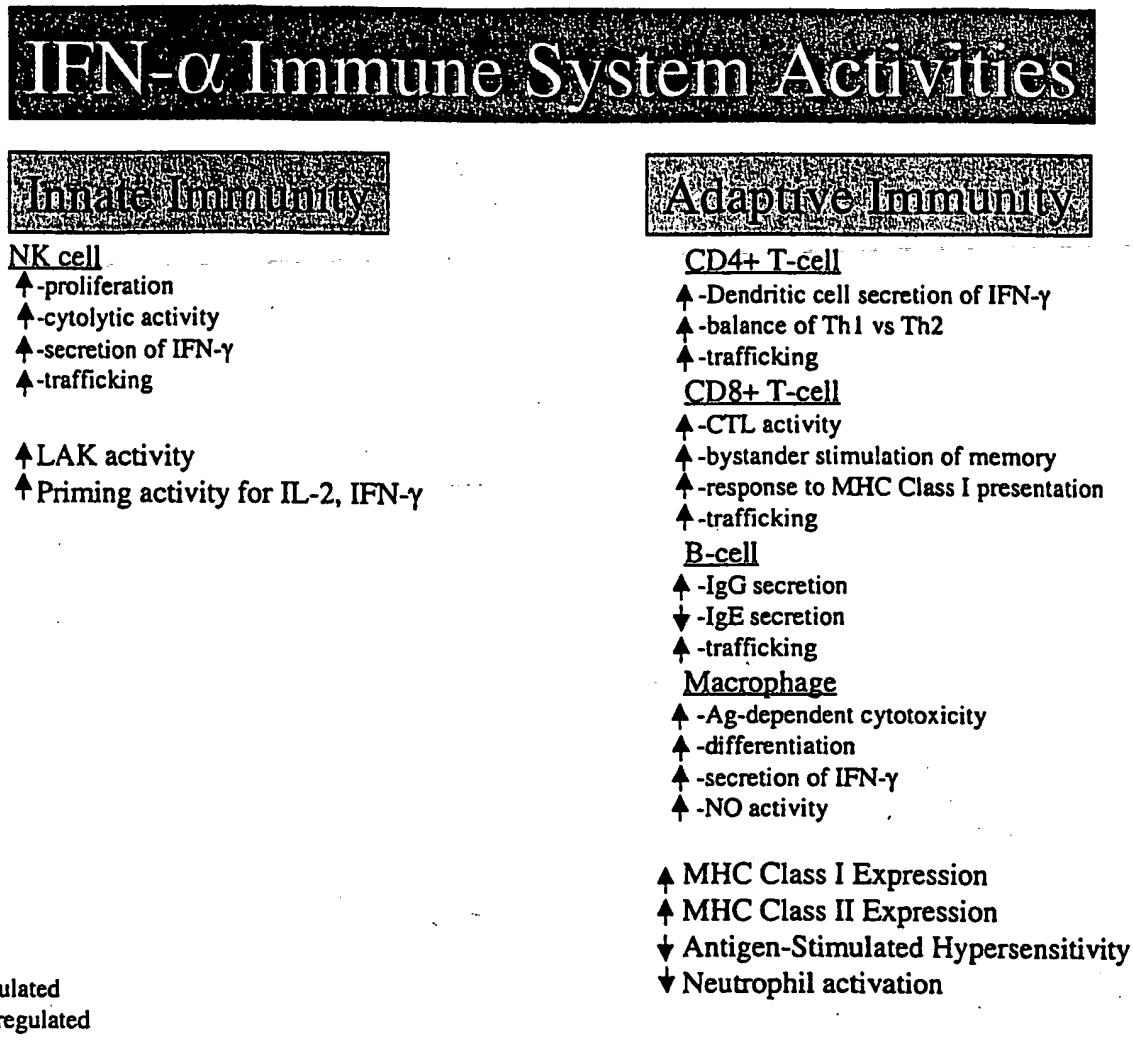

Fig. 1. IFN-α is important to the transition of the immune system from an innate response to an adaptive response. This transition is critical for the effective clearance of all nonself or foreign antigens and the maintenance of immunological memory. As shown, IFN-α affects numerous cell types including the induction of macrophage activity, NK cell cytotoxicity, and CTL activity and the decrease of neutrophil activation. These activities show overlapping function with other early response cytokines such as TGF-α and IL-2 and in some situations, can induce a synergic response.

The ability of IFN-α to prime the host immune system is consistent with the dose-dependent responses typically seen with cytokines eliciting low-dose stimulating effects and high-dose, tolerizing, or suppressant anergies [3, 23–25]. Low-dose treatment with IFN-α has been shown to down-regulate delayed-type hypersensitivity and cellular infiltration into the peripheral lymph nodes [26]. High doses of IFN-α are clinically effective against viral or neoplastic disease but are poorly tolerated by the patient [24]. In theory, low-dose treatments mimic early endogenous IFN-α priming events [27]. Studies suggest that low-dose treatment with IFN-α resembles a mucosal immune response in comparison with a systemic immune response [27]. Mucosal immunity is often referred to as local immunity, and so the distinction with IFN-α low-dose treatment is not lost. For example, the local delivery of IFN-α is responsible for NK and T-cell responsiveness to IL-12-induced secretion of IFN-γ and subsequently drives a Th1 response [19]. Additional local effects of IFN-α treatment include a block in the IL-8 attraction and activation of neutrophils at inflammation sites [23].

IFN-α links the transition of innate to adaptive immunity

IFN-α may be very important in linking the innate-immune response with the sustained adaptive-immune response [20–23, 28–30]. The innate-immune response usually consists of the cell-mediated response of NK cells to nonself (e.g., neoplastic) or foreign (e.g., viral) antigen. Although important for the initial defense of the host, the innate-immune response must transition to the more efficient and specific adaptive-immune response to clear the nonself or foreign antigen effectively. Critical to IFN-α regulation of the transition from the innate- to the adaptive-immune response is the fact that IFN-α treatment has been shown directly or indirectly to regulate the activity of other cytokines and chemokines (**Fig. 2**) [19, 23, 25]. To date, these include IFN-γ, IL-1, IL-2, IL-3, IL-6, IL-8, IL-12, IL-13, IL-15, tumor necrosis factor α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-inducible protein 10 (IP-10), and IFN-stimulated gene 15 (ISG-15) [19, 23, 31–36]. The complexity of the regulation is

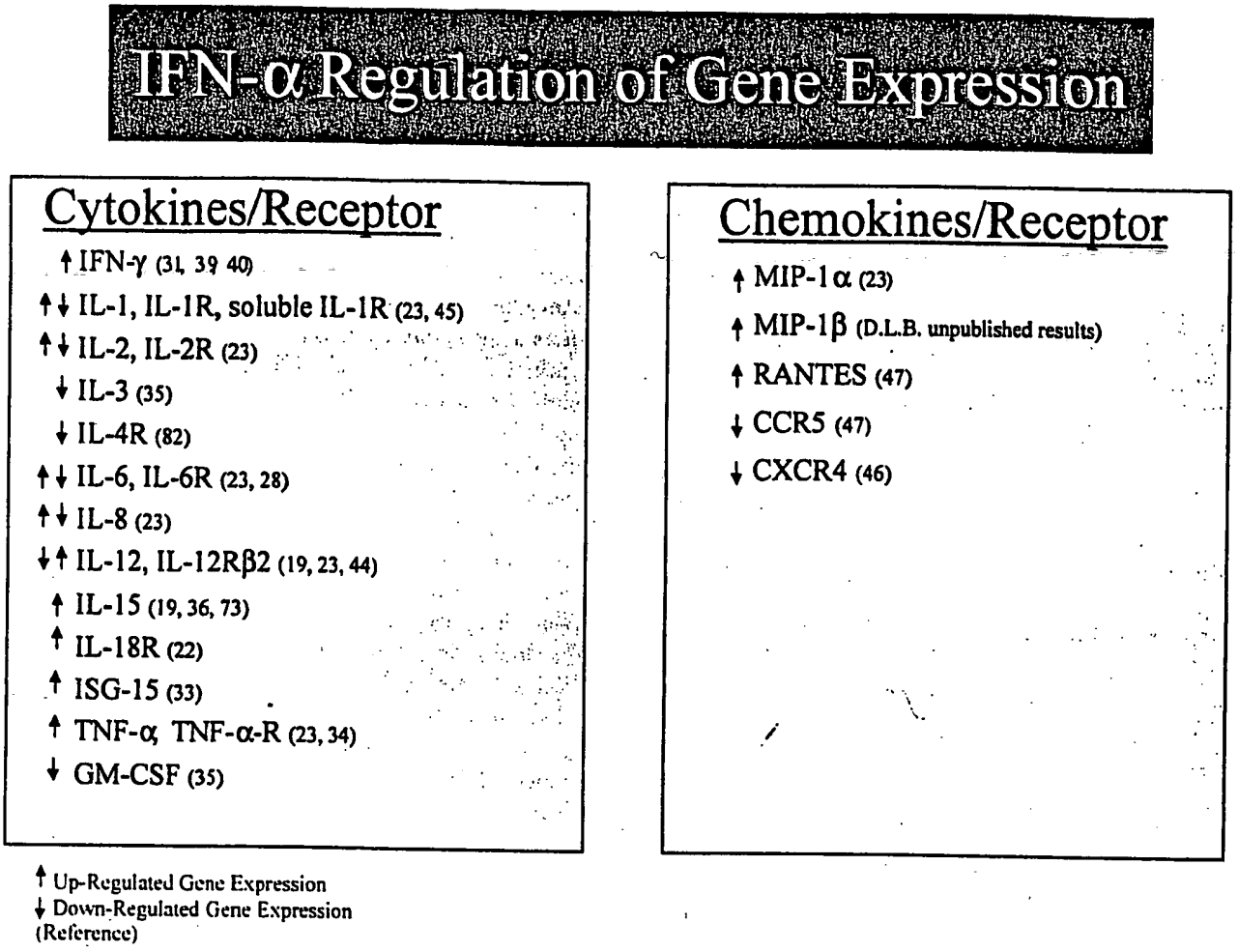

Fig. 2. As an immunomodulatory cytokine, IFN-α regulates the transcription of multiple cytokine and chemokine pathways directly or indirectly. Direct transcriptional regulation occurs after ligand binding to the type I IFN receptor and the initiation of a signal-transduction cascade, which includes members of the JAK and STAT families. Because the direct transcriptional regulation is dose-, time-, and cell-type-dependent, this emphasizes the diversity of attributable IFN-α activities and the difference between high- and low-dose therapies.

demonstrated further by a synergy between IFN-α and several other cytokines, most important including IFN-β and IFN-γ [20, 23, 37, 38]. For example, IFN-α syngergizes with IL-18 to induce IFN-γ gene transcription and protein expression [22]. In fact, a possible nexus for the innate-to-adaptive transition may be through the IFN-α-induced up-regulation of IFN-γ protein in CD4+ and CD8+ T cells and NK cells [31, 39, 40]. Furthermore, it has been suggested that IFN-α signal transduction is augmented and expanded by the signal-transduction capacity of IFN-γ [20, 37–39, 41, 42]. Despite these important signal-transduction responses, antagonism of IFN-α- and IFN-γ-induced transcriptional activation has been observed with cytokines such as IL-4 [43]. Together, these observations underscore the complexity of the immunological regulatory mechanisms for IFN-α.

Additional literature suggests that IFN-α regulation of the host immune system is not mediated solely through the regulation of other cytokines and chemokines, but through the regulation of cytokine- and chemokine-receptor expression at the cell surface [44]. For example, IFN-α up-regulates the IL-12Rβ2 subunit in CD4+ T cells and thus, increases APC responsiveness to IL-12 [23, 44]. To date, IFN-α has been shown to regulate the cell-surface expression or soluble expression of CXCR4, CCR5, TNF-α, IL-2R, IL-1R, IL-4R, IL-6R, and IL-18R components [22, 23, 28, 45–47, 82].

IFN-α regulates the immune response through the Th1/Th2 balance

The predisposition and regulation of the CD4+ Th population are important in determining how well the host responds to an infection or a neoplasm and if it is capable of sustaining an effective, antiseptic response over the length of the challenge [48]. Recently, the importance of the balance between the CD4+ Th1 and Th2 cell populations on immune-system function has been reviewed in-depth and will not be addressed thoroughly in this article [49, 50]. However, a putative role for IFN-α in the regulation of the Th1 response has been suggested. Specifically, in vivo IFN-α treatment promotes Th1 cell differentiation indirectly and re-establishes a Th1/Th2 population balance in diseases and infections that promote a Th2 cell imbalance [29, 51]. One possible mechanism is mediated through the IFN-α-induced up-regulation of IL-12R. During differentiation of human naïve T cells into the Th1 and Th2 subsets after stimulation with antigen, there is a selective

expression of the IL-12Rβ2 subunit on Th1 cells only. In fact, IL-12 and IFN-α can induce the expression of IL-12Rβ2 in Th1 cells, thus selectively promoting a Th1 response [23, 27, 44, 52]. Furthermore, IFN-γ is a key regulatory cytokine involved in the promotion and maintenance of the Th1 cell population and is secreted by CD4+ T cells induced with IFN-α. Thus, IFN-α stimulates Th1 cell development indirectly [29–31, 51, 53–55]. Although influencing the Th1 cell population positively, IFN-α also appears to suppress the Th2 cell development through the suppression of IL-4 and IL-13 gene expression [30]. The immediate effect dampens the Th2 response by blocking IL-4 protein inducible activity, thus decreasing potential antigen hypersensitivity and maximizing the innate cell-mediated response [30].

DIRECT EFFECTS OF IFN-α ON THE HOST IMMUNE SYSTEM

IFN-α direct stimulation of the innate-immune response

The most striking innate-immune activity resulting from IFN-α treatment is the direct stimulation of NK cell-mediated, cytotoxic killing activity [34, 56, 57]. NK cell cytotoxicity is important to the clinical remission of chronic myelogenous leukemia (CML) and the pathological remission of hepatitis C virus (HCV)-infected patients [58, 59]. NK cells are one of the first professional killing cells to arrive in the early antineoplastic and antiviral immune response. Because NK cell cytotoxicity is nondirected, the activity is categorized as being a part of the innate-immune response. Locally produced IFN-α stimulates increased cytotoxic killing activity in regional NK cells, and although the mechanism of action is unclear, one aspect is mediated through a direct induction of perforin mRNA expression in CD8+ and NK cells [34, 60–62] and IFN-α has two other important regulatory effects on NK cells. IFN-α stimulates the proliferation of NK cells [25, 63]. IFN-α also enhances the production or secretion of other cytokines by the NK cell through the autocrine IFN-γ loop [34, 56, 62, 64, 65]. However, it should be noted that although IFN-α stimulates NK cell cytotoxicity, it also protects normal/uninfected cells from antibody-independent cell death by decreasing their susceptibility to nonspecific cell death [60, 66, 67]. The regulatory role of IFN-α on NK and target cells underscores both concepts for IFN-α as a requisite-priming cytokine and as a local effector molecule.

IFN-α regulation of the adaptive-immune response

Following the innate response, the host must adapt the immune system efficiently against the foreign antigen to create a strong, antigen-specific, prolonged, and regulated immune response. Adaptive immunity is initiated and driven by the proper cognate presentation to T cells of nonself, foreign antigen by the class I or class II major histocompatibility complex (MHC) on APC. Specifically, antigen presentation by the class I MHC complex or by the class II complex on a B cell stimulates the cell-mediated CD8 cytotoxic T-cell response [68].

IFN-α has been shown to effect the CD8+ T-cell and B-cell adaptive-immune response [69]. Such effects extend from a profound regulatory role by IFN-α in stimulating the proliferation, activation, and generation of existing memory CD8+ cytotoxic T cells (CTLs) to the stimulation of lymphocyte-activated killer (LAK) activity [20, 42, 70, 71]. IFN-α treatment, or treatment with inducers of IFN-α, is important for the clonal expansion and survival of the memory CD8+ T-cell population in an antigen-independent manner [70, 71]. However, this type of bystander effect is not as powerful a response as that seen characteristically with antigen-specific expansion during viral infection, which logarithmically expands the population of a few specific CD8+ T-cell populations typically from 1000- to 10,000-fold [72]. Yet, the IFN-α-stimulated expansion of the entire population of CD8+ T cells is independent of T-cell receptor (TCR) activation and represents a unique mechanism of control over adaptive-immune responses [70]. The importance of IFN-α in this effect has been suggested using IFN-α/βR knockout mice. Sun et al. [5] suggest a complete absence of bystander effects upon CpG DNA challenge in vivo and in vitro. Similar bystander effects have been seen with IL-12, IL-15, IL-18, CpG, poly(I:C), and IFN-γ treatment, which suggests a common and redundant cytokine mechanism for eradication of infectious agents [5, 36, 70, 73]. This bystander activity is important to the preservation of long-term T-cell memory and would occur during intermittent viral infections that induce IFN-α expression [72]. Furthermore, type I IFNs are also known to be involved with the nonspecific maintenance of T cells through an inhibition of apoptosis and forced quiescence in the absence of antigen [74]. Thus, IFN-α assists in antigen specificity, selection, and proliferation of CD8+ T cells during the adaptive-immune response and highlights an important interaction between the nonspecific innate-immune system and the adaptive-immune system.

Another significant activity of IFN-α identified very early from in vitro studies was the up-regulation of class I and class II MHC expression [75–77]. IFN-α up-regulates the transcription of class I MHC proteins directly, resulting in increased antigen presentation, immune surveillance, and cognate cell-mediated killing to eliminate virally infected and neoplastic cells [77, 78]. The up-regulation of class I MHC by IFN-α also promotes the development of CD8+ T-cell responses [70]. IFN-α and IFN-γ up-regulate class II MHC expression, which promotes enhanced CD4+ T-cell responses and antigen presentation [76, 79].

The role of IFN-α on the B-cell-mediated, adaptive response is more subtle. The clearest effect observed has been with immunoglobulin (Ig) production. IFN-γ and IFN-α have been shown to enhance IgG production and down-regulate IgE secretion in B cells [69, 78, 80, 81]. Under the direction of IFN-α, the Ig isotype-selection process induces a predominantly IgG2a antibody-isotype response [69]. It is suggested that IFN-α down-regulates IgE secretion through the post-transcriptional down-regulation of IL-4 and the IL-4R mRNA [82]. This IFN-α activity antagonizes IL-4-driven, Th2 immune responses and may also compliment the IFN-α stimulation of macrophage antibody-dependent cytotoxicity by the stimulation IFN-γ and inducible nitric oxide synthase (iNOS) expression in macrophages [83, 84].

Because the suppression of IgE production is important to the early immune response, it has been suggested that IFN-α secretion by an APC controls crucial immune responses of B and T cells [69]. However, this is thought to be an indirect effect because maximal Ig response to IFN-α treatment was observed in B cells only when they interact with Th cells [78]. Other IFN-α-regulated B-cell effects include the induction of IL-15 mRNA expression in macrophages, which stimulate T cells indirectly via IL-15 activity [36, 73]; the enhancement of murine macrophage phagocytosis in combination with M-CSF and IL-4 [85]; and the regulation of hematopoietic differentiation of macrophage lineage DCs with potent APC activity [35].

IFN-α has also been shown to influence lymphocyte trafficking through autocrine effects by contributing to the mobilization of the adaptive-immune response [4, 11, 72]. This is important for the anti-infective and immunoregulatory function during an immune response and is also important to recruit T-cell, B-cell, and NK cell populations from bone marrow into specialized secondary areas/tissues for antigen presentation. This localization also delivers NK cell-produced cytokines to the affected area and increases the likelihood for T- and B-cell activation in a region where there are few antigen-specific cells [21, 62]. The effect promotes an adaptive immune response during primary infection and increases the likelihood of activating low-frequency antigen-specific cells [21]. Although it remains to be determined whether this IFN-α activity is direct or indirect, the IFN-γ-induced chemokine IP-10 has also been shown to promote the migration of T cells and monocytes, suggesting an indirect IFN-α effect [32].

IFN-α signal transduction and the immune response

To date, in vitro IFN-α mechanism-of-action studies have identified multiple protein components involved in a cellular signal-transduction cascade. In addition to regulating the immune system, this signal cascade also mediates the cellular response to inappropriate, uncontrolled cellular proliferation and viral infection. Important cellular components involved with the classical IFN response include the JAK kinases, STAT transcriptional regulators, IRF transcription factors, RNase L, 2′,5′ oligo A synthetase (2′,5′ OAS), and PKR, all of which have been described thoroughly elsewhere [86]. These experiments have identified an IFN response that includes transcriptional and nontranscriptional effects [86–88]. Although viral infection is a common method to induce an IFN response, cellular stress and radiation can also stimulate IFN-α protein production or IFN-regulated genes [12, 89].

Presumably because IFN-α regulates the transcription of numerous gene products positively and negatively, including transcriptional activators and other cytokines, the effect of IFN-α treatment has often been described as pleomorphic. In fact, the regulation of other cytokines highlights the difficulty in identifying IFN-α-specific/direct effects on the host immune system. Additionally, the JAK/STAT pathways are used by many other signal-transduction-effector molecules including IFN-γ [41, 90]. Thus, it is not surprising that the direct biological consequences of IFN-α treatment have frequently been difficult to elucidate. Furthermore, a number of controversial and dogmatically defined IFN-α functions and activities could be a result of cell-differentiation state, cell type, and concentration dependence [86]. Experiments using microarray and proteomics analysis have begun to examine the complex cellular response induced by IFN-α and IFN-γ treatment [91–94]. However, much of the intracellular IFN-α mechanism of action remains to be clarified.

IFN-α as an immunotherapeutic protein

Early in vitro studies focused on the antiviral effect of IFN-α using mammalian cell lines infected with a variety of viruses [e.g., influenza, encephalomyocarditis virus (EMC), vesicular stomatitis virus (VSV), and lymphocytic choriomeningitis virus (LCMV); for review, see ref. [95]]. In addition to a direct antiviral effect, these studies have shown that IFN-α had a dramatic, inhibitory effect on cellular proliferation [96]. Because most of the cell lines used for in vitro studies were transformed, these early findings lead to research focusing on the antineoplastic properties of the IFN-α. In fact, the first FDA-approved clinical indication for IFN-α was against Hairy Cell Leukemia (HCL) [97]. Important to a discussion of mechanism of action is the fact that the antiproliferative effect of IFN-α appears to be independent of its antiviral activity, suggesting distinct mechanisms for the two activities [98–100]. However, a clear mechanism of action of immune regulation by IFN-α has yet to be elucidated because of the complexity of the immune system and malignant/viral disease progression. Recent data from clinical trials with IFN-α have revealed that there is a significant component of the immune response involved in the observed antineoplastic and antiviral activity [10, 101, 102]. Thus, although each origin of clonal neoplasm or type of virus tends to have unique aspects to its respective regulation, it is our contention that the immunologic components modulated by IFN-α therapy are critical elements in conferring therapeutic efficacy in the clinic. As a result, disease resolution or viral eradication by IFN-α may have distinct requirements that balance a direct intracellular effect and an immunomodulatory effect of IFN-α (**Fig. 3**). Studies designed to elucidate the immunomodulatory activity further are important in the future development of IFN-α as a clinical immunotherapeutic.

ANTITUMOR IMMUNOLOGY

IFN-α has a greater demonstrated, overall impact for hematological malignancies than for solid tumors, and the immunological response to IFN-α treatment has been shown to be critical to a clinical antitumor effect [101]. IFN-α has a well-known antitumor activity in mouse and human malignancies and has been shown to decrease the tumorigenicity of transplanted tumor cells [42, 103]. In fact, an immunological role for IFN-α was demonstrated first with L1210 lymphoma cells that are resistant to IFN-α treatment in vitro, and in vivo IFN-α treatment in a murine model inhibited tumor development and growth [104]. The mechanism for this effect was partially a result of CD8+ CTL activity [104]. In a clinical setting, the first licensed approval for an IFN therapeutic in the United States was as a treatment against HCL; this IFN-α indication

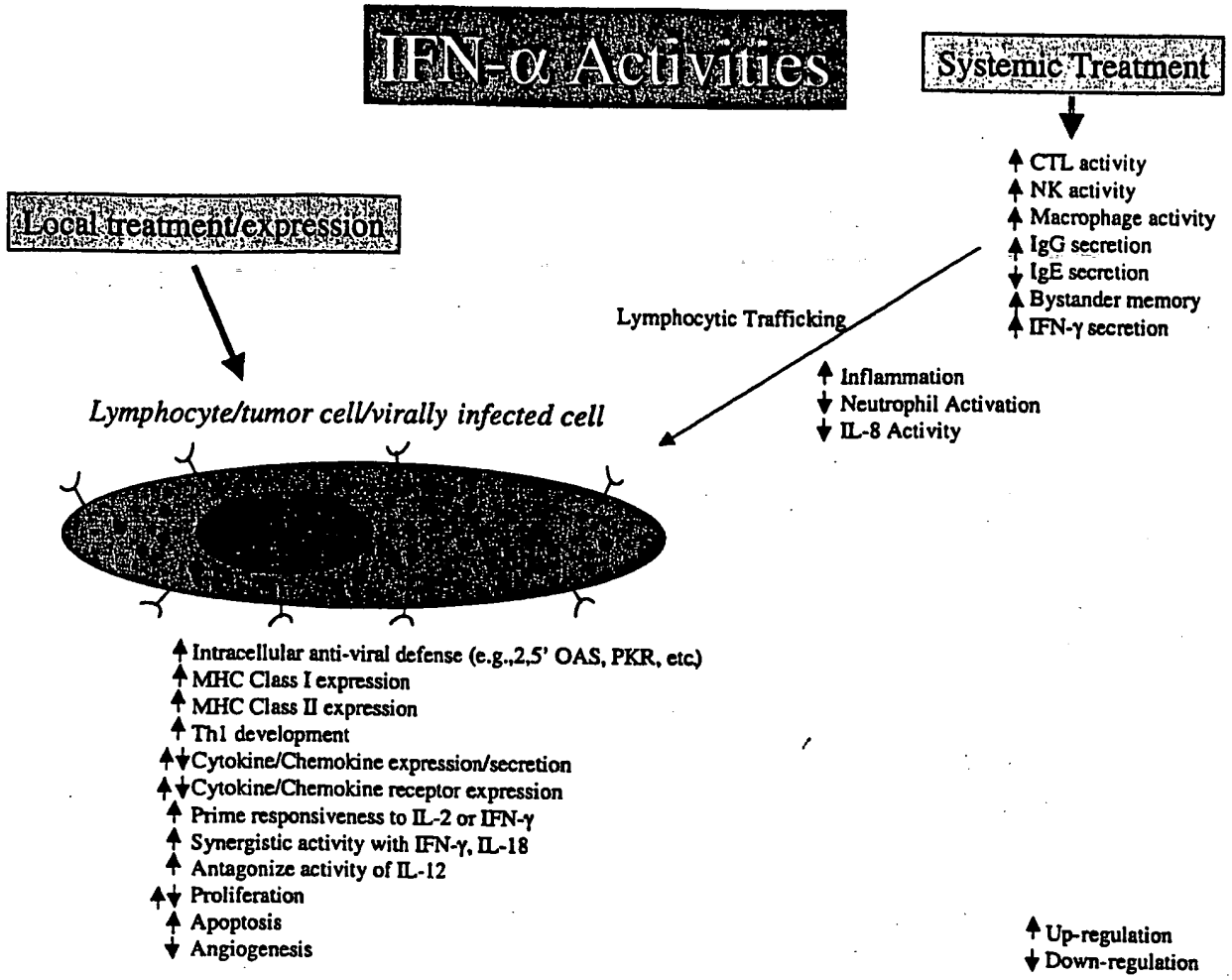

Fig. 3. As a priming cytokine, localized expression of IFN-α or low-dose treatment with IFN-α can result in a pleomorphic list of activities that range from an increase in apoptosis and immune-surveillance capability to decreased angiogenesis. As a regulator of multiple immune cells, systemic treatment with IFN-α can affect the balance of Th1 and Th2 cell populations. These activities are important to antiviral host defenses and antineoplastic disease responses. Complex intracellular signal-transduction mechanisms control these activities and result in such responses as the regulation of gene expression and cell proliferation.

was also the first FDA-approved biological therapy for a human malignancy [97]. During IFN-α treatment of HCL patients, a decreased bone marrow infiltration by malignant cells was observed and resulted in a normalization of peripheral hematologic variables and a decrease in patient morbidity [97].

Because IFNs elicit a pleomorphic effect and target the transcription of a specific subset of genes, their use and mechanism of action are unique compared with conventional, systemic chemotherapy that typically target DNA synthesis [101]. This makes combination treatment of IFN-α with standard chemotherapeutics more likely in future clinical trials, with the possibility of increasing antitumor activity compared with single-drug treatment [101]. Recent studies combining IFN-α with standard chemotherapeutics have begun to show the promise of such combination therapies [105, 106]. However, it is still unclear if the antitumor mechanism of action of IFN-α is because of direct antiproliferative or apoptosis effects, immune-system stimulation of a CTL-mediated immune response, or cytokine stimulation. Of course, a combination of these activities is likely. Future studies with IFN-α in human and animal models will be needed to address this gap in understanding.

What is known currently is that an important step to the elimination of a solid tumor is through immunosurveillance, and IFN-α plays an important role in controlling tumor growth through the regulation and proliferation of memory CD8+ T cells in a way that is not seen with IFN-γ, G-CSF, or IL-4 treatment [42]. Alterations in MHC-antigen expression are crucial to oncogenesis and metastasis development. Most tumor cells exhibit a partial or complete loss of MHC antigens on the cell surface [107, 108]. These tumor-specific effects result in a paucity of tumor antigen presented by APC DCs as a result of an absence of T-cell activity and the poor infiltration of DCs into tumors [108]. DCs play an important role in antitumor immunosurveillance as rare, potent APCs and IFN-α-producing cells [109]. The expression of nonself antigens when presented through class I MHC complexes is critical to the stimulation of an adaptive-immune response involving the CD8+ cytotoxic lymphocyte activity. Thus, although CTLs are crucial effectors to immunosurveillance, they are not normally activated effectively in tumor-bearing hosts. In fact, cancer patients show low or undetectable levels of CTL in peripheral blood, and reduced immunological cell performance is a prognostic indicator of antitumor-immune response for advanced

cervical cancer [108, 110]. Antitumor therapies that up-regulate MHC gene expression in tumor cells (including IFNs, retinoic acid, and calcitrol) are thought to induce immunologic rejection of the tumor cell. Because IFNs can also stimulate the proliferation, activation, and generation of CTLs, this also increases the likelihood of tumor-cell cytolysis [20, 70].

Although an effective antitumor response will promote persistence of CD8+ CTL activity in the midst of a productive CD4+ activity, it is very clear that CD8+ CTL activity alone cannot reduce tumor burden [108]. In addition to CD8+ CTL antitumor activity, macrophages and NK cells have important antitumor cytotoxicity [64]. In fact, tumor cells suppress the local immune response by expressing inhibitory NK cell receptors. These inhibitory receptors negatively regulate the lytic activity of tumor-specific CTLs and demonstrate the importance of NK cell activity in reducing tumor burden [111, 112].

The development of metastasis may be under the control of cytokines and chemokines released by the tumor [113–115]. Cancer cells attempt to circumvent cytokine signaling and thus evade antitumor, antigen-specific, immune cells. There is also an inverse correlation between IFN-γ expression in tumor-infiltrating lymphocytes and disease state [115]. Recent evidence suggests that IFN-γ and perforin are critical components of tumor-suppressor response of the immune system to protect against the development of carcinogen-induced sarcomas in athymic nude mice [116]. Regulation of tumor-cell immunogenicity is key to tumor evasion of an antitumor response with documented evidence of altered IFN signal-transduction-response pathways in tumorigenic cell lines [117–119]. Together, these data underscore the importance of IFNs in antitumor activity by the host immune system.

IFN regulation of the host immune system is important to its mechanism as an antitumor activity. This influence can be seen with IFN-α treatment of tumor cells, which markedly increase the transcription and expression of class I MHC antigens [107]. IFN-γ can also increase the expression of some tumor-associated antigens and has been shown to up-regulate a variety of cell-surface adhesion molecules on tumor cells and effector cells [108, 120, 121]. As with the antiviral activity of IFN-α, IFN-α therapy promotes Th1/Th2 balance in proliferative diseases that induce a Th2-favored imbalance [29, 49]. Finally, the absence of CTL activity typically observed in patients with post-transplant lymphoproliferative disorders can be restored with IFN-α therapy [122].

The antiviral effects of IFN-α treatment in Hepatitis B and Hepatitis C disease also suppress hepatocarcinoma and liver fibrosis even when complete viral-infection eradication is not achieved [123–126]. Specifically, IFN-α HCV clinical trials show a reduced incidence of morbidity or a reduction in cirrhosis complications [127, 128]. One possible IFN-α antitumor mechanism is the induction of cell-cycle arrest in primary hepatocytes in a time- and dose-dependent manner [120, 129, 130]. This cell-cycle arrest is presumably a result of the IFN-α-induced inhibition of cyclin A and B induction, which inhibits CDK2 activity [130]. IFN-α also inhibits the proliferation of hematopoietic progenitor cells from normal and CML bone marrow including myeloid, erythroid, megakaryocyte, and multilineage colony-forming cells [120]. Separation of antiproliferative and antiviral activities via target-gene responses hints at the complexity of IFN-α-induced cellular responses in antitumor and antiviral mechanisms [98–100].

Clearance of tumorigenic cells can occur not only by a direct adaptive response through CTL activity but also through a direct induction of apoptosis [131]. IFN-α has demonstrated positive and negative effects on apoptosis, highlighting a cell type, state of cell differentiation, and context dependency for the response [86, 131]. In fact, IFN-α may play a role in inducing apoptosis in hematopoietic progenitor cells undergoing uncontrolled cellular proliferation [131]. Recent data suggest that IFN-α and IFN-γ sensitize cells to apoptosis through the up-regulation of tumor cell-surface expression of the Trail and Fas [132–136]. IFN-γ modulation of Fas expression makes carcinoma cells sensitive to antigen-specific CD8+ CTL attack [136].

The establishment of a clonal neoplasm results in the unrestricted growth of the clonal mass associated with the induction of angiogenesis to provide sufficient blood flow and nutriture to the growing mass. IFNs are important for the control of tumor inflammation and inhibit immunologically induced angiogenesis, independent of the IFN antiproliferative effects [137–140]. IFN-α-induced inhibition of angiogenesis is correlated with a decrease in β-fibroblast growth factor (BFGF) and matrix metalloprotease-9 expression and blood-vessel density. These effects lead to a cessation of progressive tumor growth [141, 142]. In fact, recent studies with a mouse-bladder cancer model have suggested that daily injections of IFN-α at a low dose show a significant antitumor response in comparison with high-dose injection therapy three times per week [142]. These studies have also demonstrated that expression of IFN-β within a tumor is inversely correlated with the level of proangiogenic molecules and vascular density [114, 141, 143]. Furthermore, the IFN-regulated chemokine IP-10 is a very potent inhibitor of angiogenesis, suggesting that IFN-α regulation of angiogenesis is mediated by an indirect mechanism [138].

By potentiating and supporting the host's immunological response against neoplastic disorder or viral infection, IFN-α therapy may enhance self-vaccination. This effect occurs through the stimulation of CTL activity and an increase of nonself antigen presentation [101]. IFN-α has been suggested for use as an adjuvant to antitumor and antiviral vaccines and for use in post-surgical, high-risk disease [42, 70, 102, 144, 145]. It is thought that the IFN-α inhibition of early T-cell activation events is important to effectively suppress adjuvant arthritis in rats and down-regulate delayed-type hypersensitivity and lymph node proliferation induced by allergen presentation [26]. Furthermore, IFN-α activity is similar to that seen with the mycobacterium Bacillus Calmette-Guerin (BCG) vaccination. Both treatments show a strong effect on macrophage activity, and they activate T-cell responses and induce IFN-γ, TNF-α, and IL-1 protein expression/secretion [146–150]. BCG vaccination efficacy is not a result of a markedly local inflammatory response; it also involves a systemic immune response involving Th cell and mononuclear cells and, in fact, may be mediated by the direct up-regulation of IFN-α [151].

CML

CML malignancy is derived from an abnormal tyrosine-kinase activity that is thought to protect CML cells from apoptotic

death [101]. IFN-α uses immunological mechanisms to down-regulate CML cell growth [120, 152]. Prolonged patient survival times are observed with IFN-α treatment correlating with a normalization of hematologic indicators. CML treatment with IFN-α yields a complete hematological response in 80% of all patients [120]. Approximately 20% of all CML patients treated with IFN-α see a reduction of cells bearing the 9–22 chromosomal translocation over the course of a 12- to 24-month therapy regimen, demonstrating a complete cytogenetic response [101]. The up-regulation of NK cell activity with IFN-α treatment is correlated with clinical remission of CML, suggesting a direct immunological effect of IFN-α on the host immune system for clinical efficacy [58]. Recently, direct evidence of a role for T-cell immunity in clearance of malignant cells has been shown, correlating the presence of CML peptide-specific T cells and clinical response after IFN therapy [153]. Similar effects were seen with allogenic bone marrow-transplant therapy but not chemotherapy. These results suggest that IFN-α can induce CML remission by facilitating autologous leukemia-reactive CTL expansion [153, 154].

Because CML progenitors are unresponsive to β1-integrin-mediated inhibition of proliferation, it has been suggested that IFN-α treatment may actually reverse this responsiveness and restore the integrin regulation of cell growth and proliferation [155]. Similar effects have been seen with treatment of naïve T cells and lymphocyte proliferation in lymphoproliferative disorders where IFN-α directly inhibits [5, 120, 156]. Thus, the IFN-α mechanism of action in CML therapy minimally involves antiproliferative and immunological components and may also explain the efficacy against other lymphoproliferative disorders.

Melanoma

Maximally tolerated doses of IFN-α have been used in melanoma patients at high risk of reoccurrence after surgery [101]. In 20% of all IFN-α-treated patients, there is tumor-burden regression and reduction of reoccurrence rate [101, 157]. Preliminary data correlate therapy relapse with melanoma cell expression of nonclassical human leukocyte antigen (HLA) molecules (HLA-G) prior to IFN therapy [158]. These data suggest that IFN-α-therapy unresponsiveness might be a result of altered NK cell immunosurveillance. Additional work needs to be performed to further understand cancer patient responsiveness to IFN therapy.

In murine melanoma models and clinical trials, IFN-α therapy shows a greater antitumor activity when the tumor burden is reduced [101, 159]. Thus, the antitumor activity of IFN-α against melanoma seems to be a dose-intensive effect [101]. IFN-α alone and as an adjuvant in combination treatment with GM2 vaccine showed improvement in relapse-free survival of patients [160, 161]. These effects might be related to the adjuvant activities of IFN-α. The success of IFN-α against melanoma has led to suggestions of combination use with more traditional chemotherapeutic agents [102]. Future clinical trials will determine whether IFN-α combination therapy proves more efficacious than single-drug therapy.

ANTIVIRAL IMMUNOLOGY

IFN-α plays a very important role in the host antiviral defense by directly inhibiting the intracellular-viral lifecycle or by regulating the immune-system T-cell response during viral infection [19, 162]. IFN-α, -β, and -γ, together and separately, inhibit most stages of replication and the lifecycle of a wide variety of viruses. For example, SV40 and retroviral entry/uncoating are inhibited by IFN treatment, while influenza, VSV, and picornaviruses are inhibited by disruption of viral RNA transcription and decreased vRNA stability. Adenovirus, reovirus, and vaccinia virus have been shown to be inhibited by IFNs at the stage of viral protein translation, while retroviruses and VSV virus replication are inhibited by a block in viral particle maturation and release [86]. IFN activation of the 2′,5′ OAS and PKR proteins inhibits the cellular protein-synthesis machinery that may play an important role in inhibiting viral replication or tumor growth (for review, see refs. [21, 86]). Most importantly, although in vivo viral infection models aide in the elucidation of antiviral immunology, the mechanistic complexity of the IFN-α control of the host immune system demonstrates how many activation pathways must exist, through diverse target genes, to induce an antiviral state [8, 40, 163, 164].

Studies with IFN-α/βR$^{-/-}$ mice have yielded information regarding the regulation of the immune system by IFN-α to control viral infection. Although these mice have an otherwise normal immune system, they are unable to eradicate viral infection, have a markedly reduced NK cell activity response to infection, and lack an antiviral CD8+ T-cell CTL activity [8, 40]. Because in vivo expression of IFN-α induces an antiviral state that can protect permissive cells, IFN-α/βR$^{-/-}$ mice also show altered cell and tissue tropism during viral infection [165]. Notably, IFN-α/βR$^{-/-}$ mice are highly susceptible to viral infections despite an intact IFN-γ host immune-response pathway. Although lacking IFN-α/β induction of IFN-γ, IFN-α/βR$^{-/-}$ mice use IL-12 to regulate IFN-γ production alternatively, demonstrating a functional redundancy and plasticity within the immune system [40]. However, this functional redundancy of activating IFN-γ expression is unable to replace the loss of IFN-α/β antiviral activities. Thus, IFN-α and IFN-γ are nonredundant functionally and are essential for antiviral defense [8, 164]. In fact, although the IFN-α signal-transduction pathway is independent of IFN-γ activity, it is augmented by IFN-γ expression and the expanded signal-transduction network of IFN-γ activity [20, 37–39, 41, 42].

Because an effective adaptive-immune-response defense requires previous exposure of host to the virus, the virus can succeed in establishing an active infection of the host in the absence of an effective adaptive response. Many studies have demonstrated that in an in vivo infection, the initial response by innate-immune cells is to increase production of IFN-α/β to stimulate NK cell activity [34, 56, 57]. Recent experiments have shown that NK cell activation receptors are a component of in vivo resistance to viral infection and vital to innate-host response. This activity is based on the fact that NK cells use host activation/inhibition receptors to influence cytolytic activity [166].

Immunosurveillance of virally infected cells is an important component of the host adaptive-immune response. During a typical viral lifecycle, some of the viral proteins are transported to the cell surface as intact proteins or as peptide fragments that have been processed by the intracellular ubiquitin/proteosome/transporter in antigen processing (TAP) mechanism for presentation by the cell's MHC scaffolding. In addition to up-regulating class I MHC expression, IFN-α and IFN-γ can enhance the proteolytic processing and class I MHC presentation of viral antigens through the up-regulation of ubiquitin-conjugating enzymes, proteosome enzymes, and TAP transporter proteins [94, 167, 168]. Because ubiquitination is a rate-limiting step in antigen presentation, this means that IFNs can enhance antigen presentation by macrophages [94]. This is very important to host production of an antibody-specific cellular-cytotoxic response against the virally infected cell. When the viral antigen is presented in a cognate relationship on the infected host cell and when driven by an IFN-α-stimulated Th1 T-cell response, there is a directed, adaptive, cell-mediated response by the CD8+ CTLs. Once an antigen-specific CD8+ T cell is generated, IFN-α can stimulate its CD8+ CTL activity directly [20, 70]. Numerous virus-infection models have demonstrated that activation of CD8+ CTL is critical for clearing viremia early in a primary infection, presumably because the CD8+ T cells are capable of lysing virally infected cells in an antigen-specific, HLA-restricted way [169]. Furthermore, in vivo exposure to IFN-α during the primary immune response to a viral infection induces a nonspecific, bystander CD8+ T-cell proliferation and promotes the survival of antigen-specific and nonspecific CD8+ memory T cells [70]. Tough et al. [70] present evidence that maintenance of memory CD8+ T cells is not through TCR stimulation but rather through intermittent contact with a variety of cytokines. The maintenance of memory CD8+ T cells in the absence of antigen presentation ensures an antigen-specific immune response during future infections.

Clearance of virally infected and tumorigenic cells as a result of IFN-α treatment can occur not only through an indirect adaptive response and the activation of a CTL response but also via the direct induction of apoptosis. Recent studies have suggested that IFN-α sensitizes cells to apoptosis through many of its transcriptionally regulated genes, including IRF-1, PKR, and 2′,5′ OAS [86, 131]. Specifically, the DNA-binding activity of IRF-1 has been shown to up-regulate the transcription of Caspase 1 in T cells in response to DNA damage [170]. Although IRF-3 is not regulated transcriptionally by IFN-α, IRF-3 is activated during viral infection by an unknown kinase and is a potent inducer of apoptosis [88]. IFN-α-regulated gene products have also been linked with Trail-, Fas-, p53-, c-myc-, and Bcl-2-dependent apoptosis [133, 135, 136, 171, 172]. These IFN-α-regulated, apoptotic mechanisms are thought to be important not only for the elimination of virally infected cells but also for the elimination of activated T cells, which limits a T-cell response and is a mechanism for controlling the proinflammatory response-immune system [134].

LCMV

LCMV is a noncytopathic virus that can induce a persistent/chronic infection and has taught much about antiviral immunology in animal model systems [173]. LCMV demonstrates general and virus-specific CD8+ effects, such as the ability of virus-specific CD8+ cells to cycle/regulate cytokine secretion and maintain a steady state of perforin expression [174]. Studies in murine persistent/chronic-infection models have shown that in the absence of IFN-α, there is no CTL response to LCMV-infected cells, which results in unregulated LCMV infection [40]. LCMV studies have also demonstrated that complete exhaustion of T-cell immunity allows for the persistence of viral infection and the depletion or silencing of viral- and antigen-specific cytotoxic T cells [175–177]. Such studies show a selective deletion of epitope-specific memory CTLs following infection with heterologous/unrelated viruses [178]. These experiments have demonstrated that LCMV infection is a model system where memory T-cell populations for multiple pathogens are accommodated over the course of a host's lifetime. It is clear from in vivo studies using LCMV that the balance of CD4+ and CD8+ cell populations within a host is important [179]. In these studies, the absence of CD4+ cells results in virus-specific CD8+ effector cells that did not secrete IFN-γ and were unable to kill virally infected cells [177, 179]. This has a clear impact on the host's ability to respond to viral infection, and these findings are similar to CD4+/CD8+ T-cell codependency data derived from $CD4^{-/-}$ mice [180]. Furthermore, LCMV studies with IFN-α/$\beta R^{-/-}$ mice have demonstrated that IL-12 stimulation of IFN-γ in the absence of IFN-α is not enough to clear the viral infection [40]. Thus, although the antiviral activity of IFN-α has been shown to be synergistic with IFN-γ, together IFN-α and IFN-γ are critical components of the host immune response [78, 164].

HCV

IFN-α has been the most successful antiviral/immunological therapy for the eradication of HCV infection. A comprehensive review of HCV and the disease induced by infection can be found in ref. [181]. Furthermore, thorough reviews of HCV infection regarding genotype-specific differences and patient responders versus nonresponders will not be discussed in this review but can be found elsewhere [182, 183]. Pertinent to this review, there are immune-related dysfunctions in addition to the liver cirrhosis associated with chronic HCV infection. The immune-system dysfunction is specific to the phase of HCV infection/disease. For instance, a strong Th1 response and subsequent weak or absent Th2 response are observed in patients with acute HCV infection. This is in stark contrast to patients that develop a chronic HCV infection and show a predominant Th2 response correlated with weak Th1 activity [183]. During chronic HCV infection, CTL activity lyses HCV-infected cells, and there is a correlation between the presence of intralobular CD8+ T cells and high-serum alanine aminotransferase (ALT) levels [184].

Important to this discussion, there is a marked decrease in HCV RNA levels in patient sera after IFN-α treatment [128]. The earliest possible treatment with IFN-α is important to the dose-dependent viral-load reduction and term efficacy [185].

Recent clinical trials have revealed important IFN-α immunoregulatory effects, including a restoration of Th1/Th2 homeostasis, decreased liver necrosis, decreased inflammation, and a normalization of ALT serum levels associated with IFN-α treatment of HCV-infected individuals [128, 183]. IFN-α therapy also counteracts the proinflammatory response by increasing the circulating concentration of soluble IL-1R and thus, inhibiting IL-1 activity [45]. Other immunological effects of IFN-α treatment are the increase of macrophage- and lymphocyte-activation markers (such as CD69) after IFN-α treatment and an enhancement of NK cell cytotoxicity, which is correlated with clinical and pathological regression of chronic HCV infection [59, 162]. Clinical trial data support the hypothesis that the success of IFN-α therapy is to potentate the host's pre-existing, antiviral response, which in the absence of IFN-α, is insufficient to eradicate the viral infection [61, 186].

Although IFN-α treatment has demonstrated a decrease in HCV RNA load in a portion of the patient population, significant clinical effects have been observed in patients with chronic hepatitis C infection treated with a combination of IFN-α plus Ribavirin [183, 187], a broad-spectrum, antiviral, ribonucleoside analogue that interferes with viral transcription, inhibits ribonucleoprotein synthesis, and has been suggested to be an RNA virus mutagen [187, 188]. Clinically, the combination of IFN-α and Ribavirin is synergistic and over 24 or 48 weeks of therapy, gives overall, sustained virology response rates of 33 and 41%, respectively [183, 187]. Mechanistically, it has been suggested that IFN-α and Ribavirin treatment in HCV chronically infected patients up-regulates IL-10 and down-regulates IL-2 and IL-12 to inhibit or reduce a cytolytic inflammatory response [187]. In comparison to single-drug therapy, the combination with IFN-α and Ribavirin also appears to restore the balance between the Th1 and Th2 cell populations more quickly and improve the efficiency of a cytolytic T-cell response in HCV-infected cells [183, 187].

The pegylated modification of IFN-α (PegIFNα) results in a protein that has a longer protein half-life in patient sera and shows equivalent activity compared with the unmodified IFN-α [189]. Clinically, the antiviral activity of combined PegIFNα/Ribavirin treatment was shown to be dose-related and synergistic compared with PegIFNα monotherapy [190]. Compared with the administration of IFN-α three times weekly, once-weekly administration of PegIFNα reduced renal clearance and improved the combination efficacy associated with reducing viral load and cirrhosis complications [191, 192]. PegIFNα clinical trials suggest that key antiviral and immunological mechanisms can be modulated by prolonged exposure to IFN-α, effectively reducing the drug-dosing regimen. In comparison with IFN-α, PegIFNα therapy also demonstrates an improved Th2 down-regulation, increased macrophage activity, down-regulation of CD4/CD8 activation after viral challenge, and improved HCV antiviral efficacy [190–192].

Inhibition of HCV replication in tissue-culture conditions, where there is no immune-system response, has been shown recently [193]. One of the primary IFN-α antiviral activities that attempts to inhibit HCV replication and translation initiation is the PKR protein. As a viral defense against intracellular IFN activity, the HCV viral proteins NS5A and E2 inhibit PKR activity [194–197]. Presumably, IFN-α also inhibits HCV replication in a manner similar to that observed with hepatitis B virus (HBV)-replication inhibition, where IFN-α inhibits the production of progeny viruses by blocking the reverse-transcriptase activity of the HBV polymerase protein [86, 198]. However, because an HCV-in vitro replication system has only been developed recently, much of the mechanism of action of IFN remains to be elucidated [199, 200].

Human immunodeficiency virus (HIV)

Recently, IFN-α therapy has been suggested as a treatment for HIV-infected individuals. In this situation, IFN-α is unique in its mechanism of action when compared with highly active, antiretroviral therapy (HAART) [201]. As an antiviral and immunotherapeutic drug, it is suggested that IFN-α can boost the host's immune system in response to HIV infection. Important to its viral tropism is the fact that HIV can replicate in quiescent and stimulated cells. Because the primary host receptor for HIV is the CD4 protein, macrophages, CD4+ T cells, DCs, and monocytes are infected initially or during reinfection from a reservoir [202–208]. Given the viral tropism for CD4+ T cells, it is not surprising that numerous immunological abnormalities occur during the asymptomatic period of HIV infection [209–212]. Because pDC2s represent the "professional" IFN-producing cells and have potent APC activity important in host defense, preservation of these cells is associated with disease protection [15, 16]. Recent data presented by Soumelis et al. [213] demonstrate a negative correlation between the number of circulating, natural IFN-α-producing cells (pDC2s) in patient blood and HIV viral load. This is the first study demonstrating that pDC2s are affected during HIV infection. pDC2 blood measurements also suggest that these cells play an important role in the protection against opportunistic infections and Kaposi sarcoma. Thus, a measure of patient pDC2 blood levels could prove to be an important parameter to monitor in assessing the status of the immune system in HIV-infected patients. During HIV infection, there is a decline in the CD4+ and CD8+ T-cell populations, decreased CD4+ Th-cell activity, failed Ig response in B cells, and decreased IFN-γ production by the host immune system [209, 214–216]. The progressive loss of CD4+ T cells is presumably because of direct HIV infection and subsequent cell deletion [210, 217]. Some studies have shown that CXCR4+/CD8+ T-cell death is macrophage-dependent and enhanced during HIV infection by the direct stimulation of macrophage cytotoxicity by the HIV gp120 protein [218]. However, the mechanisms regulating the premature turnover of CD4+ and CD8+ T cells are still not well-elucidated.

Important to this discussion is the fact that long-term nonprogressors maintain an antiviral HIV Th-cell response during infection. In HIV-infected individuals, the disruption of the adaptive immune response correlates with an increase in viral load, a decline in CD8+ CTL activity, and AIDS progression [219–222]. Recent studies have shown that there is a concomitant increase in circulating IL-7 levels, which is indicative of a homeostatic increase of IL-7 by DCs in response to T-cell depletion [223]. As has been seen in murine LCMV models, the loss of the CD4+ T-cell population negatively impacts the ability of the host to clear the viremia [48, 224, 225]. Furthermore, Champagne et al. [212] have shown that there is a

skewed maturation of HIV-specific CD8+ T cells during HIV infection with an accumulation of preterminally differentiated memory T cells, possibly as a result of the rapid turnover of terminally differentiated CD8+ T cells. Apparently, this effect is HIV-specific, because it was not seen in CMV-infected patients.

As was demonstrated with LCMV studies, CD8+ T cells play a critical role in controlling HIV viremia [220, 226–229]. This is supported by in vivo animal experiments that show that in $CD4^{-/-}$ mice, the CD8+ T cells have normal cytotoxic activity but there is a greatly reduced development of class II MHC-restricted Th-cell activity [180]. In fact, two antiviral activities of CD8+ T cells have been described during HIV infection. The first mechanism involves direct cytolysis of HIV-infected cells in an antigen-specific, HLA-restrictive manner [220, 230]. The second mechanism involves the secretion of soluble factors [including the chemokines macrophage-inflammatory protein (MIP)-1α, MIP-1β, and regulated on activation, normal T expressed and secreted (RANTES)], which antagonize HIV binding to the CCR5 coreceptor [231, 232]. CD8+ T-cell secretion of CCR5 agonists inhibits R5-HIV binding to its host coreceptor, CCR5, and suppresses post-entry viral replication by down-regulating transcription in HIV-infected cells. This inhibition occurs without inducing cell death [47, 222, 224, 226, 229, 233–237].

During HIV infection, the host immune system makes virus-specific CTLs from epitopes within the Env, Gag, Pol, Nef, Tet, Tat, and Rev HIV proteins [220, 221, 230, 238, 239]. Rosenberg et al. [224] have demonstrated that the systemic control of HIV viral load was associated with an HIV-specific CD4+ T-cell response to the p24 protein. As the HIV infection progresses, there is a deletion or silencing of CTL populations specific for the gag, p24, and NP epitopes [221, 239]. Furthermore, rapid AIDS progressors elicit only a transient Gag-specific CTL response that correlates to an apparent inability to control viral replication and spread [239]. Although these "unresponsive" CD8+ CTL cells have TCR signal recognition, they do not proliferate in vitro [240]. These effects result in decreased HIV-antigen exposure or recognition and thus, decreased immunosurveillance capability.

It has been firmly established that HIV binding and entry into a host cell are dependent on the CD4 receptor and the chemokine coreceptors CCR5 and CXCR4 [231, 241]. However, given the dramatic loss of CD8+ T cells during HIV infection, a direct infection of CD8+ T cells by HIV has been suggested [206, 242]. Recent analysis of chronically infected patient sera has detected HIV isolates that infect T lymphocytes independent of CD4 binding, suggesting that their primary host receptor is actually the CD8 protein [243, 244]. The significance of this finding has to be more thoroughly examined to determine the percentage of CD8-specific HIV isolates in the patient population. Although a direct HIV infection might explain the loss or depletion of CD8+ T cells observed in HIV patients, there is also strong evidence that the primary method for reduction of the CD8+ population is the macrophage-mediated apoptosis of HIV-infected and uninfected CD8+ T cells [218]. These literature inconsistencies remain to be clarified.

There are many ways that HIV-1 infection attempts to control the host immune system and block IFN intracellular, antiviral activity. Similar to scenarios seen with HCV infection, the HIV-1 Tat protein has demonstrated anti-IFN activity by inhibiting PKR activity directly [245, 246]. Meanwhile the HIV-1 Nef protein down-regulates class I MHC expression at the cell surface by delaying transport from the endoplasmic reticulum to the plasma membrane. This effect down-regulates antigen presentation by the HIV-infected cell [247]. Furthermore, like most retroviruses that down-regulate expression of their respective receptor, HIV also down-regulates the cell-surface expression of the CD4 protein through the activity of the viral Nef protein [248]. This may have a significant impact on the normal host cell, signal-transduction cascades.

This emphasizes the potential, positive influence of IFN-α treatment in HIV-infected individuals, given its proven regulation of the adaptive-immune response. In vitro studies with IFN-α treatment of HIV-infected cells have shown a suppression of virion production during early stages of viral replication [249–254]. Type 1 IFNs also been shown to down-regulate CCR5 and CXCR4 chemokine-receptor expression on T-cell surfaces [46, 47]. Finally, IFN-α is thought to protect a cell from viral infection and protect viral antigen-specific T-cell clones. In fact, in vitro experiments with HIV-1-specific T-cell clones, which are eliminated typically during HIV-1 infection, are protected with IFN-α treatment [255]. In theory, in vivo treatment with IFN-α can reverse or overcome most of these HIV infection-specific, negative effects on the host immune response. As has been seen with HAART, decreasing viral load can reverse HIV-driven, CD4+ T-cell defects in AIDS patients [201]. In fact, recent phase II clinical trials with PEG-Intron have shown a 0.5-log decrease in viral titer during Peg-Intron treatment (unpublished results). It will be important to determine if in an HIV clinical trial, IFN-α counteracts proinflammatory cytokines such as IL-1, as was seen with IFN-α therapy in chronic HCV-infected individuals [45]. Thus, future clinical trials with IFN-α treatment of HIV-infected individuals may expand our knowledge of IFN-α regulation of the immune system in immunocompetent and immunocompromised patients.

CONCLUSION

The IFN-α mechanism of action as an antineoplastic and antiviral therapy has been elucidated slowly in the past 20 years because of the inherent complexity of the immune-system response to neoplastic and viral disease. Given these difficulties, it is understandable that the first-identified, IFN-α activities were direct, intracellular effects involving the inhibition of viral replication. However, even the direct antiproliferative/proapoptotic IFN-α activity is important to immune-cell function and plays a role in regulating the host immune response to disease. Furthermore, IFN-α acts directly and indirectly on many cell functions in the immune response to neoplasm and viral infection, including the induction of important downstream cytokines such as IFN-γ. As an early response cytokine, IFN-α is poised as a key priming cytokine for the immune antineoplastic and antiviral response. Local effects of

IFN-α expression include the activation of an immediate and effective innate-immune response. IFN-α then plays a critical role in directing the transition from innate to adaptive immunity through a variety of mechanisms including the control of host Th1/Th2 responses and the regulation of CD8+ CTL activity and memory. The expanding body of preclinical experience with IFN-α suggests that immunomodulation plays a significant role in mediating the therapeutic effects exhibited in clinical trials. However, to date, an explanation for the mechanism of action of IFN-α in various disease states remains theoretical but clearly deserving of further study. Given the complexity of IFN-α regulation cited herein, much of the work will have to come from animal studies in which the immune system can be manipulated. Unfortunately, many of the diseases for which IFN-α is indicated are not modeled easily in animals. Thus, additional disease models and future clinical trials will be needed to elucidate the role of IFN-α in immune-system regulation.

REFERENCES

1. Isaacs, A., Lindenman, J. (1957) Virus interference: the interferon. Proc. R. Soc. Med. 147, 258–267.
2. Havell, E. A., Hayes, T. G., Vilcek, J. (1978) Synthesis of two distinct interferons by human fibroblasts. Virology 89, 330–334.
3. Havell, E. A., Spitalny, G. L. (1983) Endotoxin-induced interferon synthesis in macrophage cultures. J. Reticuloendothel. Soc. 33, 369–380.
4. Komgold, R., Blank, K. J., Murasko, D. M. (1983) Effect of interferon on thoracic duct lymphocyte output: induction with either poly I:poly C or vaccinia virus. J. Immunol. 130, 2236–2240.
5. Sun, S., Zhang, X., Tough, D. F., Sprent, J. (1998) Type I interferon-mediated stimulation of T cells by CpG DNA. J. Exp. Med. 188, 2335–2342.
6. Wheelock, F. (1965) Interferon like virus inhibitor induced in human leukocytes by phytohemagglutin. Science 149, 310–311.
7. Rady, P. L., Cadet, P., Bui, T. K., Tyring, S. K., Baron, S., Stanton, G. J., Hughes, T. K. (1995) Production of interferon gamma messenger RNA by cells of non-immune origin. Cytokine 7, 793–798.
8. Muller, U., Steinhoff, U., Reis, L. F. L., Hemmi, S., Pavlovic, J., Zinkernagel, R. M., Aguet, M. (1994) Functional role of Type I and Type II interferons in antiviral defense. Science 264, 1918–1921.
9. Vilcek, J., Sen, G. C. (1996) Interferons and other cytokines. In *Fundamental Virology* (B. N. Fields, D. M. Knipe, P. M. Howley, eds.), Philadelphia, Lippincott-Raven, 341–365.
10. Bogdan, C. (2000) The function of type I interferons in antimicrobial immunity. Curr. Opin. Immunol. 12, 419–424.
11. Ishikawa, R., Biron, C. A. (1993) IFN induction and associated changes in splenic leukocyte distribution. J. Immunol. 150, 3713–3727.
12. Francis, M. L., Fan, X. S., Meltzer, M. S. (1996) Loss ability to produce IFN-alpha in response to HIV-1 as monocytes differentiate into macrophages. Induction through a mechanism independent of double-stranded RNA. J. Immunol. 156, 2481–2487.
13. Foster, G. R., Finter, N. B. (1998) Are all type I human interferons equivalent? J. Viral. Hepat. 5, 143–152.
14. Cederblad, B., Alm, G. V. (1990) Infrequent but efficient interferon-α-producing human mononuclear leukocytes induced by herpes simplex virus in vitro studied by immuno-plaque and limiting dilution assays. J. Interferon Res. 10, 65–73.
15. Ferbas, J. J., Toso, J. F., Logar, A. J., Navratil, J. S., Rinaldo, C. R. (1994) CD4+ blood dendritic cells are potent producers of IFN-alpha in response to in vitro HIV-1 infection. J. Immunol. 152, 4649–4662.
16. Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., Antonenko, S., Liu, Y-J. (1999) The nature of the principal type 1 interferon-producing cells in human blood. Science 284, 1835–1837.
17. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y-J., Pulendran, B., Palucka, K. (2000) Immunobiology of dendritic cells. Annu. Rev. Immunol. 18, 767–811.
18. Beilharz, M. W., McDonald, W., Watson, M. W., Heng, J., McGeachie, J., Lawson, C. M. (1997) Low-dose oral type I interferons reduce early virus replication of murine cytomegalovirus in vivo. J. Interferon Cytokine Res. 17, 625–630.
19. Biron, C. A. (1999) Initial and innate responses to viral infections—pattern setting in immunity or disease. Curr. Opin. Microbiol. 2, 374–381.
20. von Hoegen, P. (1995) Synergistic role of type I interferons in the induction of protective cytotoxic T lymphocytes. Immunol. Lett. 47, 157–162.
21. Biron, C. A. (1998) Role of early cytokines, including alpha and beta interferons (IFN-alpha/beta), in innate and adaptive immune responses to viral infections. Semin. Immunol. 10, 383–390.
22. Sareneva, T., Julkunen, I., Matikainen, S. (2000) IFN-alpha and IL-12 induce IL-18 receptor gene expression in human NK and T cells. J. Immunol. 165, 1933–1938.
23. Taylor, J. L., Groesberg, S. E. (1998) The effects of interferon-alpha on the production and action of other cytokines. Semin. Oncol. 25, 23–29.
24. Tovey, M. G., Meritet, J. P., Guymarho, J., Maury, C. (1999) Mucosal cytokine therapy: marked antiviral and antitumor activity. J. Interferon Cytokine Res. 19, 911–921.
25. Biron, C. A. (2001) Interferons alpha and beta as immune regulators—a new look. Immunity 14, 661–664.
26. Yoshino, S. (1996) Effects of oral administration of type I interferon on adjuvant arthritis in rats. Comp. Immunol. Microbiol. Infect. Dis. 19, 133–138.
27. Tompkins, W. A. (1999) Immunomodulation and therapeutic effects of the oral use of interferon-alpha: mechanism of action. J. Interferon Cytokine Res. 19, 817–828.
28. Anthes, J. C., Zhan, Z., Gilchrest, H., Egan, R. W., Siegel, M. I., Billah, M. M. (1995) Interferon-alpha down-regulates the interleukin-6 receptor in a human multiple myeloma cell line, U266. Biochem. J. 309, 175–180.
29. Faro, A. (1998) Interferon-alpha and its effects on post-transplant lymphoproliferative disorders. Springer Semin. Immunopathol. 20, 425–436.
30. Dickensheets, H. L., Donnelly, H. L. (1999) Inhibition of IL-4-inducible gene expression in human monocytes by type I and type II interferons. J. Leukoc. Biol. 65, 307–312.
31. Brinkmann, V., Geiger, T., Alkan, S., Heusser, C. H. (1993) Interferon alpha increases the frequency of interferon gamma-producing human CD4+ T cells. J. Exp. Med. 178, 1655–1663.
32. Taub, D. D., Lloyd, A. R., Conlon, K., Wang, J. M., Harada, A., Matsushima, K., Kelvin, D. J., Oppenheim, J. J. (1993) Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. J. Exp. Med. 177, 1809–1814.
33. D'Cunha, J., Ramanujam, S., Wagner, R. J., Witt, P. L., Knight Jr., E., Borden, E. C. (1996) In vitro and in vivo secretion of human ISG15, an IFN-induced immunomodulatory cytokine. J. Immunol. 157, 4100–4108.
34. Mori, S., Jewett, A., Cavalcanti, M., Murakami-Mori, K., Nakamura, S., Bonavida, B. (1998) Differential regulation of human NK cell-associated gene expression following activation by IL-2, IFN-alpha and PMA/ionomycin. Int. J. Oncol. 12, 1165–1170.
35. Tretter, T., Aman, M. J., Bug, G., Huber, C., Peschel, C. (1998) Hematopoietic growth factors are differentially regulated in monocytes and CD4+ T lymphocytes: influence of IFN-alpha and interleukin-4. J. Interferon Cytokine Res. 18, 95–102.
36. Zhang, X., Sun.S., Hwang, I., Tough, D. F., Sprent, J. (1998) Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity 8, 591–599.
37. Levy, D. E., Lew, D. J., Decker, T., Kessler, D. S., Darnell, J. J. E. (1990) Synergistic interaction between interferon-alpha and interferon-gamma through induced synthesis of one subunit of the transcription factor ISGF3. EMBO J. 9, 1105–1111.
38. Improta, T., Pine, R., Pfeffer, L. M. (1992) Interferon-gamma potentiates the antiviral activity and the expression of interferon-stimulated genes induced by interferon-alpha in U937 cells. J. Interferon Cytokine Res. 12, 87–94.
39. Hunter, C. A., Gabriel, K. E., Radzanowski, T., Neyer, L. E., Remington, J. S. (1997) Type I interferons enhance production of IFN-gamma by NK cells. Immunol. Lett. 59, 1–5.
40. Cousens, L. P., Peterson, R., Hsu, S., Dorner, A., Altman, J. D., Ahmed, R., Biron, C. A. (1999) Two roads diverged: interferon alpha/beta- and interleukin 12-mediated pathways in promoting T cell interferon gamma responses during viral infection. J. Exp. Med. 189, 1315–1328.
41. Bandyopadhyay, S. K., Kalvakolanu, D. V. R., Sen, G. C. (1990) Gene induction by interferons: functional complementation between trans-activing factors induced by alpha interferon and gamma interferon. Mol. Cell. Biol. 10, 5055–5063.

42. Belardelli, F., Ferrantini, M., Santini, S. M., Baccarini, S., Proietti, E., Colombo, M. P., Tough, D. F., Sprent, J. (1998) The induction of in vivo proliferation of long-lived CD44hi CD8+ T cells after the injection of tumor cells expressing IFN-alpha1 into syngeneic mice. Cancer Res. 58, 5795–5802.
43. Lamer, A. C., Petricoin, E. F., Nakagawa, Y., Finbloom, D. S. (1993) IL-4 attenuates the transcriptional activation of both IFN-alpha and IFN-gamma-induced cellular gene expression in monocytes and monocytic cell lines. J. Immunol. 150, 1944–1950.
44. Rogge, L., Barberis-Maino, L., Biffi, M., Passini, N., Presky, D. H., Gubler, U., Sinigaglia, F. (1997) Selective expression of an interleukin-12 receptor component by human T helper 1 cells. J. Exp. Med. 185, 825–831.
45. Naveau, S., Emilie, D., Borotto, E., Portier, A., Lazizi, Y., Giraud, V., Grangeot-Keros, L., Capron, F., Galanaud, P., Chaput, J. C. (1997) Interleukin-1 receptor antagonist plasma concentration is specifically increased by alpha-2A-interferon treatment. J. Hepatol. 27, 272–275.
46. Shirazi, Y., Pitha, P. M. (1998) Interferon downregulates CXCR4 (fusin) gene expression in peripheral blood mononuclear cells. J. Human Virol. 1, 69–76.
47. Cremer, I., Vieillard, V., De Maeyer, E. (2000) Retrovirally mediated IFN-beta transduction of macrophages induces resistance to HIV, correlated with up-regulation of RANTES production and down-regulation of C-C chemokine receptor-5 expression. J. Immunol. 164, 1582–1587.
48. Barouch, D. H., Santra, S., Schmitz, J. E., Kuroda, M. J., Fu, T. M., Wagner, W., Bilska, M., Craiu, A., Zheng, X. X., Krivulka, G. R., Beaudry, K., Lifton, M. A., Nickerson, C. E., Trigona, W. L., Punt, K., Freed, D. C., Guan, L., Dubey, S., Casimiro, D., Simon, A., Davies, M. E., Chastain, M., Strom, T. B., Gelman, R. S., Montefiori, D. C., Lewis, M. G., Emini, E. A., Shiver, J. W., Letvin, N. L. (2000) Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science 290, 486–492.
49. Miossec, P. (1997) Cytokine-induced autoimmune disorders. Drug Saf. 17, 93–104.
50. Yates, A., Bergmann, C., Van Hemmen, J. L., Stark, J., Callard, R. (2000) Cytokine-modulated regulation of helper T cell populations. J. Theo. Biol. 206, 539–560.
51. Wenner, C. A., Guler, M. L., Macatonia, S. E., O'Garra, A., Murphy, K. M. (1996) Roles of IFN-gamma and IFN-alpha in IL-12-induced T helper cell-1 development. J. Immunol. 156, 1442–1447.
52. Gallagher, R. (1997) Tagging T cells: Th1 or Th2? Science 275, 1615.
53. Pernis, A., Gupta, S., Gollob, K. J., Garfein, E., Coffman, R. L., Schindler, C., Rothman, P. (1995) Lack of interferon gamma receptor beta chain and the prevention of interferon gamma signaling in TH1 cells. Science 269, 245–247.
54. Vieillard, V., Cremer, I., Lauret, E., Rozenbaum, W., Debre, P., Autran, B., De Maeyer, L. (1997) Interferon beta transduction of peripheral blood lymphocytes from HIV-infected donors increases Th1-type cytokine production and improves the proliferative response to recall antigens. Proc. Natl. Acad. Sci. USA 94, 11595–11600.
55. Sareneva, T., Matikainen, S., Kurimoto, M., Julkunen, I. (1998) Influenza A virus-induced IFN-alpha/beta and IL-18 synergistically enhance IFN-gamma gene expression in human T cells. J. Immunol. 160, 6032–6038.
56. Ortaldo, J. R., Phillips, W., Wasserman, K., Herberman, R. B. (1980) Effects of metabolic inhibitors on spontaneous and interferon-boosted human natural killer cell activity. J. Immunol. 125, 1839–1844.
57. Li, B. L., Xiao, X. X., Liu, X. Y., Kim, H. S., Raska, K., Ortaldo, J. R., Schwartz, B., Pestka, S. (1990) Alpha-interferon structure and natural killer cell stimulatory activity. Cancer Res. 50, 5328–5332.
58. Meseri, A., Delwail, V., Mahon, F. X., Pelletier, D., Guilhot, F., Brizard, A., Gombert, J., Tanzer, J., Goube de Laforest, P. (1991) Natural-killer cell activity and cytogenetic response in chronic myelogenous leukemia treated with alpha-interferon. Br. J. Haematol. 78, 585–586.
59. Bonavita, M. S., Franco, A., Paroli, M., Santilio, I., Benvenuto, R., De Petrillo, G., Levrero, M., Perrone, A., Balsano, C., Buonaiuto, V. (1993) Normalization of depressed natural killer activity after interferon-alpha therapy is associated with a low frequency of relapse in patients with chronic hepatitis C. Int. J. Tissue React. 15, 11–16.
60. Trinchieri, G., Santoli, D., Granato, D., Perussia, B. (1981) Antagonistic effects of interferons on the cytotoxicity mediated by natural killer cells. Fed. Proc. 40, 2705–2710.
61. Balian, A., Naveau, S., Zou, W., Durand-Gasselin, I., Bouchet, I., Foussat, A., Galanaud, P., Chaput, J. C., Emilie, D. (2000) Pretreatment expression of the perforin gene by circulating CD8(+) T lymphocytes predicts biochemical response to interferon-alpha in patients with chronic hepatitis C. Eur. Cytokine Netw. 11, 177–184.
62. Salazar-Mather, T. P., Ishikawa, R., Biron, C. A. (1996) NK cell trafficking and cytokine expression in splenic compartments after IFN induction and viral infection. J. Immunol. 157, 3054–3064.
63. Biron, C. A., Sonnenfeld, G., Welsh, R. M. (1984) Interferon induces natural killer cell blastogenesis in vivo. J. Leukoc. Biol. 35, 31–37.
64. Herberman, R. B., Holden, H. T. (1979) Natural killer cells as antitumor effector cells. J. Natl. Cancer Inst. 62, 441–445.
65. Ortaldo, J. R., Mantovani, A., Hobbs, D., Rubenstein, M., Pestka, S., Herberman, R. B. (1983) Effects of several species of human leukocyte interferon on cytotoxic activity of NK cells and monocytes. Int. J. Cancer 31, 285–289.
66. Einhorn, S., Blomgren, H., Cantell, K., Strander, H. (1979) Effect if prolonged in vivo administration of leukocyte interferon on the mitogen responsiveness of human lymphocytes. Acta Med. Scand. 206, 345–350.
67. Einhorn, S., Blomgren, H., Strander, H. (1979) Interferon and spontaneous cytotoxicity in man. Effect of interferon on lymphocytes and target cells in vitro. Cancer Lett. 7, 1–7.
68. Germain, R. N. (1995) The biochemistry and cell biology of antigen presentation by MHC class I and class II molecules. Implications for development of combination vaccines. Ann. N. Y. Acad. Sci. 754, 114–125.
69. Finkelman, F. D., Svetic, A., Gresser, I., Snapper, C., Holmes, J., Trotta, P. P., Katona, I. M., Gause, W. C. (1991) Regulation by interferon alpha of immunoglobulin isotype selection and lymphokine production in mice. J. Exp. Med. 174, 1179–1188.
70. Tough, D. F., Borrow, P., Sprent, J. (1996) Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science 272, 1947–1950.
71. Parronchi, P., Mohapatra, S., Sampognaro, S., Giannarini, L., Wahn, U., Chong, P., Mohapatra, S., Maggi, E., Renz, H., Romagnani, S. (1996) Effect of interferon-α on cytokine profile, T cell receptor repertoire and peptide reactivity of human allergen-specific T cells. Eur. J. Immunol. 26, 697–703.
72. Ahmed, R. (1996) Tickling memory T cells. Science, 272, 1904.
73. Tough, D. F., Sun, S., Zhang, X., Sprent, J. (2000) Stimulation of memory T cells by cytokines. Vaccine 18, 1642–1648.
74. Akbar, A. N., Lord, J. M., Salmon, M. (2000) IFN-α and IFN-β: a link between immune memory and chronic inflammation. Immunol. Today 21, 337–342.
75. Pignatelli, M., Waters, J., Lever, A., Iwarson, S., Schaff, Z., Gerety, R., Thomas, H. C. (1986) HLA class I antigens on the hepatocyte membrane during recovery from acute hepatitis B virus infection and during interferon therapy in chronic hepatitis B virus infection. Hepatology 6, 349–353.
76. Rhodes, J., Ivanyi, J., Cozens, P. (1986) Antigen presentation by human monocytes: effects of modifying major histocompatibility complex class II antigen expression and interleukin 1 production by using recombinant interferons and corticosteroids. Eur. J. Immunol. 16, 370–375.
77. Fellous, M., Nir, U., Wallach, D., Merlin, G., Rubenstein, M., Revel, M. (1982) Interferon-dependent induction of mRNA for the major histocompatibility antigens in human fibroblasts and lymphoblastoid cells. Proc. Natl. Acad. Sci. USA 79, 3082–3086.
78. Neubauer, R. H., Goldstein, L., Rabin, H., Stebbing, N. (1985) Stimulation of in vitro immunoglobulin production by interferon-alpha. J. Immunol. 134, 299–304.
79. Steimle, V., Siegrist, C. A., Mottet, A., Lisowska-Grospierre, B., Mach, B. (1994) Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science 265, 106–109.
80. Pene, J., Rousset, F., Briere, F., Chretien, I., Bonnefoy, J. Y., Spits, H., Yokota, T., Arai, N., Arai, K., Banchereau, J. (1988) IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc. Natl. Acad. Sci. USA 85, 6880–6884.
81. Finkelman, F. D., Holmes, J., Katona, I. M., Urban Jr., J. F., Beckmann, M. P., Park, L. S., Schooley, K. A., Coffman, R. L., Mosmann, T. R., Paul, W. E. (1990) Lymphokine control of in vivo immunoglobulin isotype selection. Annu. Rev. Immunol. 8, 303–333.
82. So, E. Y., Park, H. H., Lee, C. E. (2000) IFN-gamma and IFN-alpha posttranscriptionally down-regulate the IL-4-induced IL-4 receptor gene expression. J. Immunol. 165, 5472–5479.
83. Karupiah, G., Xie, Q. W., Buller, R. M., Nathan, C., Duarte, C., MacMicking, J. D. (1993) Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. Science 261, 1445–1448.
84. Kamijo, R., Harada, H., Matsuyama, T., Bosland, M., Gerecitano, J., Shapiro, D., Le, J., Koh, S. I., Kimura, T., Green, S. J., et al. (1994) Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. Science 263, 1612–1615.

85. Sampson, L. L., Heuser, J., Brown, E. J. (1991) Cytokine regulation of complement receptor-mediated ingestion by mouse peritoneal macrophages. M-CSF and IL-4 activate phagocytosis by a common mechanism requiring autostimulation by IFN-beta. J. Immunol. 146, 1005–1013.
86. Stark, G. R., Kerr, I. M., Agarwala, S. S., Williams, B. R., Silverman, R. H., Schreiber, R. D. (1998) How cells respond to interferons. Annu. Rev. Biochem 67, 227–264.
87. Horvath, C. M., Darnell, J. J. E. (1996) The antiviral state induced by alpha interferon and gamma interferon requires transcriptionally active Stat1 protein. J. Virol. 70, 647–650.
88. Smith, E. J., Marie, I., Prakash, A., Garcia-Sastre, A., Levy, D. E. (2001) IRF3 and IRF7 phosphorylation in virus-infected cells does not require double-stranded RNA-dependent protein kinase R or Ikappa B kinase but is blocked by vaccinia virus E3L protein. J. Biol. Chem. 276, 8951–8957.
89. Clave, E., Carosella, E. D., Gluckman, E., Socie, G. (1997) Radiation-enhanced expression of interferon-inducible genes in the KG1a primitive hematopoietic cell line. Leukemia 11, 114–119.
90. Foster, G. R. (1997) Interferons in host defense. Semin. Liver Dis. 17, 287–295.
91. Celis, J. E., Justesen, J., Madsen, P. S., Lovmand, J., Pedersen Ratz, G., Celis, A. (1987) Major proteins induced and down-regulated by interferons in human cultured cells: identification of a unique set of proteins induced by interferon-alpha in epithelial, fibroblast, and lymphoid cells. Leukemia 1, 800–813.
92. Der, S. D., Zhou, A., Williams, B. R., Silverman, R. H. (1998) Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Proc. Natl. Acad. Sci. USA 95, 15623–15628.
93. Aboagye-Mathiesen, G., Ebbesen, P., von der Maase, H., Celis, J. E. (1999) Interferon gamma regulates a unique set of proteins in fresh human bladder transitional cell carcinomas. Electrophoresis 20, 344–348.
94. Nyman, T. A., Matikainen, S., Sareneva, T., Julkunen, I., Kalkkinen, N. (2000) Proteome analysis reveals ubiquitin-conjugating enzymes to be a new family of interferon-alpha-regulated genes. Euro. J. Biochem. 267, 4011–4019.
95. Joklik, W. K. (1991) Interferons. In *Fundamental Virology* (B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, B. Roizman, eds.), New York, Raven, 343–370.
96. Balkwill, F., Oliver, R. T. (1977) Growth inhibitory effects of interferon on normal and malignant human haemopoietic cells. Int. J. Cancer 20, 500–505.
97. Pfeffer, L. M., Dinarell, C. A., Herberman, R. B., Williams, B. R., Borden, E. C., Bordens, R., Walter, M. R., Nagabhushan, T. L., Trotta, P. P., Pestka, S. (1998) Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. Cancer Res. 58, 2489–2499.
98. van Heuvel, M., Govaert-Siemerink, M., Bosveld, I. J., Zwarthoff, E. C., Trapman, J. (1988) Interferon-alpha-(IFN) producing CHO cell lines are resistant to the antiproliferative activity of IFN: a correlation with gene expression. J. Cell Biochem. 38, 269–278.
99. Deblandre, G. A., Marinx, O. P., Evans, S. S., Majjaj, S., Leo, O., Caput, D., Huez, G. A., Wathelet, M. G. (1995) Expression cloning of an interferon-inducible 17-kDa membrane protein implicated in the control of cell growth. J. Biol. Chem. 270, 23860–23866.
100. Arora, T., Floyd-Smith, G., Espy, M. J., Jelinek, D. F. (1999) Dissociation between IFN-alpha-induced anti-viral and growth signaling pathways. J. Immunol. 162, 3289–3297.
101. Borden, E. C. (1998) Gene regulation and clinical roles for interferons in neoplastic diseases. Oncologist 3, 198–203.
102. Agarwala, S. S., Kirkwood, J. M. (2000) Update on the role of adjuvant interferon for high risk melanoma. Trends Exp. Clin. Med. 10, 230–239.
103. Gutterman, J. U. (1994) Cytokine therapeutics: lessons from interferon alpha. Proc. Natl. Acad. Sci. USA 91, 1198–1205.
104. Gresser, I., Maury, C., Brouty-Boye, D. (1972) Mechanism of the anti-tumour effect of interferon in mice. Nature 239, 167–168.
105. Verastegui-Aviles, E., Mohar, A., Mota, A., Guadarrama, A., De La Garza-Salazar, J. (1999) Combination of radiation therapy and interferon alpha-2b in patients with advanced cervical carcinoma: a pilot study. Int. J. Gynecol. Cancer 9, 401–405.
106. Miyamoto, A., Umeshita, K., Sakon, M., Nagano, H., Eguchi, H., Kishimoto, S., Dono, K., Nakamori, S., Gotoh, M., Monden, M. (2000) Advanced hepatocellular carcinoma with distant metastases, successfully treated by a combination therapy of alpha-interferon and oral tegafur/uracil. J. Gastroenterol. Hepatol. 15, 1447–1451.
107. Harris, H. W., Gill, T. J. (1986) Expression of class I transplantation antigens. Transplantation 42, 109–117.
108. Schuler, G., Kampgen, E. (1999) Vaccine therapy of malignant melanoma. Dermatol. Ther. 10, 62–73.
109. Kochman, S., Bernard, J. (1999) Antitumour immune response and cancer vaccination: the critical role of dendritic cells. Curr. Med. Res. Opin. 15, 321–326.
110. Marana, H. R., Silva, J. S., Andrade, J. M., Bighetti, S. (2000) Reduced immunologic cell performance as a prognostic parameter for advanced cervical cancer. Int. J. Gynecol. Cancer 10, 67–73.
111. Cati, A., Guerra, N., Giron-Michel, J., Azzarone, B., Angevin, E., Moretta, A., Chouaib, S., Caignard, A. (2001) Tumor cells regulate the lytic activity of tumor-specific cytotoxic t lymphocytes by modulating the inhibitory natural killer receptor function. Cancer Res. 61, 3240–3244.
112. Morretta, A., Bottino, C., Vitale, M., Pende, D., Cantoni, C., Mingari, M. C., Biassoni, R., Morretta, L. (2001) Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu. Rev. Immunol. 19, 197–223.
113. Negus, R. P., Balkwill, F. (1996) Cytokines in tumour growth, migration and metastasis. World J. Urol. 14, 157–165.
114. Fidler, I. J. (2000) Regulation of neoplastic angiogenesis. J. Natl. Cancer Inst. Monogr. 28, 10–14.
115. Csiszar, A., Szentes, T., Haraszti, B., Zou, W., Emilie, D., Petranyi, G., Pocsik, E. (2001) Characterisation of cytokine mRNA expression in tumour-infiltrating mononuclear cells and tumour cells freshly isolated from human colorectal carcinomas. Eur. Cytokine Netw. 12, 87–96.
116. Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., Old, L. J., Schreiber, R. D. (2001) IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 410, 1107–1111.
117. Petricoin, E. F., David, M., Fang, H., Grimley, P., Larner, A. C., Vande Pol, S. (1994) Human cancer cell lines express a negative transcriptional regulator of the interferon regulatory factor family of DNA binding proteins. Mol. Cell. Biol. 14, 1477–1486.
118. Matsuura, H., Sakaue, M., Subbaramaiah, K., Kamitani, H., Eling, T. E., Dannenberg, A. J., Tanabe, T., Inoue, H., Arata, J., Jetten, A. M. (1999) Regulation of cyclooxygenase-2 by interferon gamma and transforming growth factor alpha in normal human epidermal keratinocytes and squamous carcinoma cells. Role of mitogen-activated protein kinases. J. Biol. Chem. 274, 29138–29148.
119. Dovhey, S. E., Ghosh, N. S., Wright, K. L. (2000) Loss of interferon-γ inducibility of TAP1 and TAP2 in a renal cell carcinoma cell line. Cancer Res. 60, 5789–5796.
120. Guilhot, F., Lacotte-Thierry, L. (1998) Interferon-alpha: mechanisms of action in chronic myelogenous leukemia in chronic phase. Hematol. Cell Ther. 40, 237–239.
121. Kaplan, D. H., Shankaran, V., Dighe, A. S., Stockert, E., Schreiber, R. D. (1998) Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc. Natl. Acad. Sci. USA 95, 7556–7561.
122. Taguchi, T., Purtilo, D. T., Okano, M. (1994) The effect of intravenous immunoglobulin and interferon-alpha on Epstein-Barr virus-induced lymphoproliferative disorder in a liver transplant recipient. Transplantation 57, 1813–1815.
123. Manabe, N., Chevallier, M., Chossegros, P., Causse, X., Guerret, S., Trepo, C., Grimaud, J. A. (1993) Interferon-alpha 2b therapy reduces liver fibrosis in chronic non-A, non-B hepatitis: a quantitative histological evaluation. Hepatology 18, 1344–1349.
124. Nishiguchi, S., Kuroki, T., Nakatani, S., Morimoto, H., Takeda, T., Nakajima, S., Shiomi, S., Seki, S., Kobayashi, K., Otani, S. (1995) Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 346, 1051–1055.
125. Duchatelle, V., Marcellin, P., Giostra, L., Bregeaud, L., Pouteau, M., Boyer, N., Auperin, A., Erlinger, S., Henin, D., Degott, C. (1998) Changes in liver fibrosis at the end of alpha interferon therapy and 6 to 18 months later in patients with chronic hepatitis C: quantitative assessment by a morphometric method. J. Hepatol. 29, 20–28.
126. Nishiguchi, S., Shiomi, S., Nakatani, S., Takeda, T., Fukuda, K., Tamori, A., Habu, D., Tanaka, T. (2001) Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. Lancet 357, 196–197.
127. Fattovich, G., Giustina, G., Degos, F., Diodati, G., Tremolada, F., Nevens, F., Alamsio, P., Solinas, A., Brouwer, J. T., Thomas, H., et al. (1997) Effectiveness of interferon alfa on incidence of hepatocellular carcinoma and decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J. Hepatol. 27, 201–205.
128. Poynard, T., Moussalli, J., Ratziu, V., Regimbeau, C., Opolon, P. (1999) Effect of interferon therapy on the natural history of hepatitis C virus-

related cirrhosis and hepatocellular carcinoma. Clin. Liver Dis. 3, 869–881.
129. Balkwill, F., Taylor-Papadimitriou, J. (1978) Interferon affects both G1 and S+G2 in cells stimulated from quiescence to growth. Nature 274, 798–800.
130. Murphy, D., Detjen, K. M., Welzel, M., Wiedenmann, B., Rosewicz, S. (2001) Interferon-alpha delays S-phase progression in human hepatocellular carcinoma cells via inhibition of specific cyclin-dependent kinases. Hepatology 33, 346–356.
131. Barber, G. N. (2000) The interferons and cell death: guardians of the cell or accomplices of apoptosis? Semin. Cancer Biol. 10, 103–111.
132. Egle, A., Villunger, A., Kos, M., Bock, G., Gruber, J., Auer, B., Greil, R. (1996) Modulation of Apo-1/Fas (CD95)-induced programmed cell death in myeloma cells by interferon-alpha 2. Eur. J. Immunol. 26, 3119–3126.
133. Ugurel, S., Seiter, S., Rappl, G., Stark, A., Tilgen, W., Reinhold, U. (1999) Heterogenous susceptibility to CD95-induced apoptosis in melanoma cells correlates with bcl-2 and bcl-x expression and is sensitive to modulation by interferon-gamma. Int. J. Cancer 82, 727–736.
134. Kaser, A., Enrich, B., Ludwiczek, O., Vogel, W., Tilg, H. (1999) Interferon-alpha (IFN-alpha) enhances cytotoxicity in healthy volunteers and chronic hepatitis C infection mainly by the perforin pathway. Clin. Exp. Immunol. 118, 71–77.
135. Kayagaki, N., Yamaguchi, N., Nakayama, M., Eto, H., Okumura, K., Yagita, H. (1999) Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: a novel mechanism for the antitumor effects of type I IFNs. J. Exp. Med. 189, 1451–1460.
136. Bergmann-Leitner, E. S., Abrams, S. I. (2000) Influence of interferon gamma on modulation of Fas expression by human colon carcinoma cells and their subsequent sensitivity to antigen-specific CD8+ cytotoxic T lymphocyte attack. Cancer Immunol. Immunother. 49, 193–207.
137. Sidky, Y. A., Borden, E. C. (1987) Inhibition of angiogenesis by interferons: effects on tumor- and lymphocyte-induced vascular responses. Cancer Res. 47, 5155–5161.
138. Angiolillo, A. L., Sgardi, C., Taub, D. D., Liao, F., Farber, J. M., Maheshwari, S., Kleinman, H. K., Reaman, G. H., Tosato, G. (1995) Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. J. Exp. Med. 182, 155–162.
139. Coughlin, C. M., Salhany, K. E., Gee, M. S., LaTemple, D. C., Kotenko, S., Ma, X., Gri, G., Wysocka, M., Kim, J. E., Liu, L., Liao, F., Farber, J. M., Pestka, S., Trinchieri, G., Lee, W. M. (1998) Tumor cell responses to IFNgamma affect tumorigenicity and response to IL-12 therapy and antiangiogenesis. Immunity 9, 25–34.
140. Carmeliet, P., Jain, R. K. (2000) Angiogenesis in cancer and other diseases. Nature 407, 249–257.
141. Dinney, C. P. N., Bielenberg, D. R., Perrotte, P., Reich, R., Eve, B. Y., Bucana, C. D., Fidler, I. J. (1998) Inhibition of basic fibroblast growth factor expression, angiogenesis, and growth of human bladder carcinoma in mice by systemic interferon-alpha administration. Cancer Res. 58, 808–814.
142. Slaton, J. W., Perrotte, P., Inoue, K., Dinney, C. P. N., Fidler, I. J. (1999) Interferon-alpha-mediated down-regulation of angiogenesis-related genes and therapy of bladder cancer are dependent on optimization of biological dose and schedule. Clin. Cancer Res. 5, 2726–2734.
143. Kuniyasu, H., Yasui, W., Shinohara, H., Yano, S., Ellis, L. M., Wilson, M. R., Bucana, C. D., Rikita, T., Tahara, E., Fidler, I. J. (2000) Induction of angiogenesis by hyperplastic colonic mucosa adjacent to colon cancer. Amer. J. Pathol. 157, 1523–1535.
144. Kyburz, D., Aichele, P., Speiser, D. E., Hengartner, H., Zinkernagel, R. M., Pircher, H. (1993) T cell immunity after a viral infection versus T cell tolerance induced by soluble viral peptides. Eur. J. Immunol. 23, 1956–1962.
145. Pizzocaro, G., Piva, L., Colavita, M., Ferri, S., Artusi, R., Boracchi, P., Parmiani, G., Marubini, E. (2001) Interferon adjuvant to radical nephrectomy in Robson stages II and III renal cell carcinoma: a multicentric randomized study. J. Clin. Oncol. 19, 425–431.
146. Ruco, L. P., Meltzer, M. S. (1977) Macrophage activation for tumor cytotoxicity: induction of tumoricidal macrophages by supernatants of PPD-stimulated Bacillus Calmette-Guerin-immune spleen cell cultures. J. Immunol. 119, 889–896.
147. Zwilling, B. S., Campolito, L. B. (1977) Destruction of tumor cells by BCG-activated alqolar macrophages. J. Immunol. 119, 838–841.
148. Papilian-Todoratiu, C., Risca, R., Mulea, R., Daicoviciu, D. (1990) The effect of BCG-activated macrophages on the B-16 melanoma. Morphol. Embryol. 36, 129–134.
149. Ravn, P., Boesen, H., Pedersen, B. K., Andersen, P. (1997) Human T cell responses induced by vaccination with Mycobacterium bovis bacillus Calmette-Guerin. J. Immunol. 158, 1949–1955.
150. Ibsen, M. W., Bakken, V., Jonsson, R., Hordnes, K. (1997) Immune responses in mice after gastric and subcutaneous immunization with BCG. Scand. J. Immunol. 46, 274–280.
151. Chung, J. Y., Lee, E. S., Lee, W. J., Kim, H. H., Min, K. J., Lee, C. (1993) Analysis of the immunologic mechanism of intravesical bacillus Calmette-Guerin therapy for superficial bladder tumors: distribution and function of immune cells. J. Korean Med. Sci. 8, 135–144.
152. Fujii, S. (2000) Role of interferon-alpha and clonally expanded T cells in the immunotherapy of chronic myelogenous leukemia. Leuk. Lymphoma 38, 21–38.
153. Molldrem, J. J., Lee, P. P., Wang, C., Felio, K., Kantarjian, H. M., Champlin, R. E., Davis, M. M. (2000) Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat. Med. 6, 1018–1023.
154. Fujii, S. (2000) Role of interferon-α and clonally expanded T cells in the immunotherapy of chronic myelogenous leukemia. Leuk. Lymphoma 38, 21–38.
155. Bhatia, R., McCarthy, J. B., Verfaillie, C. M. (1996) Interferon-alpha restores normal beta 1 integrin-mediated inhibition of hematopoietic progenitor proliferation by the marrow microenvironment in chronic myelogenous leukemia. Blood 87, 3883–3891.
156. Petricoin, E. F., Ito, S., Williams, B. L., Audet, S., Stancato, L. F., Camero, A., Clouse, K., Grimley, P., Weiss, A., Beeler, J., Finbloom, D. S., Shores, E. W., Abraham, R., Larner, A. C. (1997) Antiproliferative action of interferon-alpha requires components of T-cell-receptor signalling. Nature 390, 629–632.
157. Lafuma, A., Dreno, B., Delaunay, M., Emery, C., Fagnani, F., Hieke, K., Bonerandi, J. J., Grob, J. J. (2001) Economic analysis of adjuvant therapy with interferon alpha-2a in stage II malignant melanoma. Eur. J. Cancer 37, 369–375.
158. Wagner, S. N., Rebmann, V., Willers, C. P., Gross-Wilde, H., Goos, M. (2000) Expression analysis of classic and non-classic HLA molecules before interferon alfa-2b treatment of melanoma. Lancet 356, 220–221.
159. Habib, F. A., Lobocki, C., Ezhuthachan, R., Chelladurai, M., Preventza, O., Mittal, V. K. (2001) Interferon alpha2b inhibits the murine melanoma cell line Cloudman S91 in vivo but not in vitro: a model for studying tumor cell-cytokine interactions. Am. Surg. 67, 257–260.
160. Kirkwood, J. M., Ibrahim, J., Lawson, D. H., Atkins, M. B., Agarwala, S. S., Collins, K. L., Mascari, R., Morrissey, D. M., Chapman, P. B. (2001) High-dose interferon alfa-2b does not diminish antibody response to GM2 vaccination in patients with resected melanoma: results of the Multicenter Eastern Cooperative Oncology Group Phase II Trial E2696. J. Clin. Oncol. 19, 1430–1436.
161. Kirkwood, J. M., Ibrahim, J., Sosman, J. A., Sondak, V. K., Agarwala, S. S., Ernstoff, M. S., Rao, U. (2001) High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the gm2-klh/qs-21 vaccine in patients with resected stage iib-iii melanoma: results of intergroup trial e1694/s9512/c509801. J. Clin. Oncol. 19, 2370–2380.
162. Lukaszewski, R. A., Brooks, T. J. G. (2000) Pegylated alpha interferon is an effective treatment for virulent venezuelan equine encephalitis virus and has profound effects on the host immune response to infection. J. Virol. 74, 5006–5015.
163. Kimura, T., Nakayama, K., Penninger, J., Kitagawa, M., Harada, H., Matsuyama, T., Tanaka, N., Kamijo, R., Vilcek, J., Mak, T. W., et al. (1994) Involvement of IRF-1 transcription factor in antiviral responses to interferons. Science 264, 1921–1924.
164. van den Broek, M. F., Muller, U., Huang, S., Zinkernagel, R. M., Aguet, M. (1995) Immune defence in mice lacking type I and/or type II interferon receptors. Immunol. Rev. 148, 5–18.
165. Ryman, K. D., Klimstra, W. B., Nguyen, K. B., Biron, C. A., Johnston, R. E. (2000) Alpha/beta interferon protects adult mice from fatal Sindbis virus infection and is an important determinant of cell and tissue tropism. J. Virol. 74, 3366–3378.
166. Brown, M. G., Dokun, A. O., Heusel, J. W., Smith, H. R., Beckman, D. L., Blattenberger, E. A., Dubbelde, C. E., Stone, L. R., Scalzo, A. A., Yokoyama, W. M. (2001) Vital involvement of a natural killer cell activation receptor in resistance to viral infection. Science 292, 934–937.
167. Akiyama, K., Yokota, K., Kagawa, S., Shimura, N., Tamura, T., Akioka, H., Nothwang, H. G., Noda, C., Tanaka, K., Ichihara, A. (1994) cDNA cloning and interferon gamma down-regulation of proteasomal subunits X and Y. Science 265, 1231–1234.
168. Boehm, U., Klamp, T., Groot, M., Howard, J. C. (1997) Cellular responses to interferon-gamma. Annu. Rev. Immunol. 15, 749–795.

169. Doherty, P. C. (1996) Cytotoxic T cell effector and memory function in viral immunity. Curr. Top. Microbiol. Immunol. 206, 1–14.
170. Tamura, T., Ishihara, M., Lamphier, M. S., Tanaka, N., Oishi, I., Aizawa, S., Matsuyama, T., Mak, T. W., Taki, S., Taniguichi, T. (1995) An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. Nature 376, 596–599.
171. Lee, S. B., Rodriquez, D., Rodriguez, J. R., Esteban, M. (1997) The apoptosis pathway triggered by the interferon-induced protein kinase PKR requires the third basic domain, initiates upstream of Bcl-2, and involves ICE-like proteases. Virology 231, 81–88.
172. D'Souza, S., Xin, H., Walter, S., Choubey, D. (2001) The gene encoding p202, an interferon-inducible negative regulator of the p53 tumor suppressor, is a target of p53-mediated transcriptional repression. J. Biol. Chem. 276, 298–305.
173. Zinkernagel, R. M. (1996) Immunology taught by viruses. Science 271, 173–178.
174. Slifka, M. K., Rodriguez, F., Whitton, J. L. (1999) Rapid on/off cycling of cytokine production by virus-specific CD8+ T cells. Nature 401, 76–79.
175. Moskophidis, D., Lechner, F., Pircher, H., Zinkernagel, R. M. (1993) Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 362, 758–761.
176. Doherty, P. C. (1993) Immune exhaustion: driving virus-specific CD8+ T cells to death. Trends Microbiol. 1, 207–209.
177. Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J. D., Suresh, M., Altman, J. D. (1998) Viral immune evasion due to persistence of activated T cells without effector function. J. Exp. Med. 188, 2205–2213.
178. Selin, L. K., Lin, M. Y., Kraemer, K. A., Pardoll, D. M., Schneck, J. P., Varga, S. M., Santolucito, P. A., Pinto, A. K., Welsh, R. M. (1999) Attrition of T cell memory: selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. Immunity 11, 733–742.
179. Tishon, A., Lewicki, H., Rall, G., Von Herrath, M., Oldstone, M. B. (1995) An essential role for type 1 interferon-gamma in terminating persistent viral infection. Virology 212, 244–250.
180. Rahemtulla, A., Fung-Leung, W. P., Schilham, M. W., Kundig, T. M., Sambhara, S. R., Narendran, A., Arabian, A., Wakeham, A., Paige, C. J., Zinkernagel, R. M. (1991) Normal development and function of CD8+ cells but markedly decreased helper cell activity in mice lacking CD4. Nature 353, 180–184.
181. Lauer, G. M. Walker, B. D. (2001) Hepatitis C virus infection. N. Engl. J. Med. 345, 41–52.
182. Purcell, R. (1997) The hepatitis C virus: overview. Hepatology 26, 11S–14S.
183. Boyer, N., Marcellin, P. (2000) Pathogenesis, diagnosis and management of hepatitis C. J. Hepatol. 32, 98–112.
184. Hayata, T., Nakano, Y., Yoshizawa, K., Sodeyama, T., Kiyosawa, K. (1991) Effects of interferon on intrahepatic human leukocyte antigens and lymphocyte subsets in patients with chronic hepatitis B and C. Hepatology 13, 1022–1028.
185. Brouwer, J. T., Nevens, F., Kleter, B., Elewaut, A., Adler, M., Brenard, R., Chamuleau, R. A., Michielsen, P. P., Pirotte, J., Hautekeete, M. L., Weber, J., Bourgeois, N., Hansen, B. E., Bronkhorst, C. M., ten Kate, F. J., Heijtink, R. A., Fevery, J., Schalm, S. W. (1998) Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J. Hepatol. 28, 951–959.
186. Nelson, D. R., Marousis, C. G., Ohno, T., Davis, G. L., Lau, J. Y. (1998) Intrahepatic hepatitis C virus-specific cytotoxic T lymphocyte activity and response to interferon alfa therapy in chronic hepatitis C. Hepatology 28, 225–230.
187. Souvignet, C., Zarski, J-P. (2000) Combination treatment for chronic hepatitis C: what is the role of ribavirin? Fundam. Clin. Pharmacol. 14, 321–325.
188. Crotty, S., Maag, D., Arnold, J. J., Zhong, W., Lau, J. Y., Hong, Z., Andino, R., Cameron, C. E. (2000) The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. Nat. Med. 6, 1375–1379.
189. Grace, M., Youngster, S., Xie, L., Westreich, L., Jacobs, S., Brassard, D., Bausch, J., Bordens, R. (2001) Structural and biological characterization of pegylated recombinant interferon alfa-2b. J. Interferon Cytokine Res. 12, 1103–1115.
190. Glue, P., Rouzier-Panis, R., Raffanel, C., Sabo, R., Gupta, S. K., Salfi, M., Jacobs, S., Clement, R. P. (2000) A dose-ranging study of pegylated interferon alfa-2b and ribavirin in chronic hepatitis C. The Hepatitis C Intervention Therapy Group. Hepatology 32, 647–653.
191. Heathcote, E. J., Shiffman, M. L., Cooksley, W. G. E., Dusheiko, G. M., Lee, S. S., Balart, L., Reindollar, R., Reddy, R. K., Wright, T. L., Lin, A., Hoffman, J., De Pamphilis, J. (2000) Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N. Engl. J. Med. 343, 1673–1680.
192. Reddy, R. K., Wright, T. L., Pockros, P. J., Shiffman, M. L., Everson, G., Reindollar, R., Fried, M. W., Purdum, P. P., Jensen, D., Smith, C., Lee, W. M., Boyer, T. D., Lin, A., Pedder, S., DePamphilis, J. (2001) Efficacy and safety of pegylated (40-kd) interferon-2a compared with interferon-2a in noncirrhotic patients with chronic hepatitis C. Hepatology 33, 433–438.
193. Chung, R. T., He, W., Saquib, A., Contreras, A. M., Xavier, R. J., Chawla, A., Wang, T. C., Schmidt, E. V. (2001) Hepatitis C virus replication is directly inhibited by IFN-alpha in a full-length binary expression system. Proc. Natl. Acad. Sci. USA 98, 9847–9852.
194. Taylor, D. R., Shi, S. T., Romano, P. R., Barber, G. N., Lai, M. M. (1999) Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science 285, 107–110.
195. Gerotto, M., Dal Pero, F., Pontisso, P., Noventa, F., Gatta, A., Alberti, A. (2000) Two PKR inhibitor HCV proteins correlate with early but not sustained response to interferon. Gastroenterology 119, 1649–1655.
196. Berg, T., Mas Marques, A., Hohne, M., Wiedenmann, B., Hopf, U., Schreier, E. (2000) Mutations in the E2-PePHD and NS5A region of hepatitis C virus type 1 and the dynamics of hepatitis C viremia decline during interferon alfa treatment. Hepatology 32, 1386–1395.
197. Enomoto, N., Sakuma, I., Asahina, Y., Kurosaki, M., Murakami, T., Yamamoto, C., Ogura, Y., Izumi, N., Marumo, F., Sato, C. (2001) Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N. Engl. J. Med. 334, 77–81.
198. Hayashi, Y., Koike, K. (1989) Interferon inhibits hepatitis B virus replication in a stable expression system of transfected viral DNA. J. Virol. 63, 2936–2940.
199. Lohmann, V., Korner, F., Koch, J., Herian, U., Theilmann, L., Bartenschlager, R. (1999) Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110–113.
200. Pietschmann, T., Lohmann, V., Rutter, G., Kurpanek, K., Bartenschlager, R. (2001) Characterization of cell lines carrying self-replicating hepatitis C virus RNAs. J. Virol. 75, 1252–1264.
201. Autran, B., Carcelain, G., Li, T. S., Blanc, C., Mathez, D., Tubiana, R., Katlama, C., Debre, P., Leibowitch, J. (1997) Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 277, 112–116.
202. Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeshkpour, G. H., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M. A., Fauci, A. S. (1986) Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 233, 1089–1093.
203. Wiley, C. A., Schrier, R. D., Nelson, J. A., Lampert, P. W., Oldstone, M. B. (1986) Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA 83, 7089–7093.
204. Embretson, J., Zupancic, M., Ribas, J. L., Burke, A., Racz, P., Tenner-Racz, K., Haase, A. T. (1993) Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 362, 359–362.
205. Stevenson, M., Gendelman, H. E. (1994) Cellular and viral determinants that regulate HIV-1 infection in macrophages. J. Leukoc. Biol. 56, 278–288.
206. Livingstone, W. J., Moore, M., Innes, D., Bell, J. E., Simmonds, P. (1996) Frequent infection of peripheral blood CD8-positive T-lymphocytes with HIV-1. Edinburgh Heterosexual Transmission Study Group. Lancet 348, 649–654.
207. Pope, M., Betjes, M. G., Romani, N., Hirmand, H., Cameron, P. U., Hoffman, L., Gezelter, S., Schuler, G., Steinman, R. M. (1994) Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell 78, 389–398.
208. Swingler, S., Mann, A., Jacque, J., Brichacek, B., Sasseville, V. G., Williams, K., Luckner, A. A., Janoff, E. N., Wang, R., Fisher, D., Stevenson, M. (1999) HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat. Med. 5, 997–1103.
209. Miedema, F., Petit, A. J., Terpstra, F. G., Schattenkerk, J. K., de Wolf, F., Al, B. J., Roos, M., Lange, J. M., Danner, S. A., Goudsmit, J. (1988) Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. J. Clin. Investig. 82, 1908–1914.
210. Margolick, J. B., Munoz, A., Donnenberg, A. D., Park, L. P., Galai, N., Giorgi, J. V., O'Gorman, M. R., Ferbas, J. J. (1995) Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. The Multicenter AIDS Cohort Study. Nat. Med. 1, 674–680.

211. Roederer, M. (1995) T-cell dynamics of immunodeficiency. Nat. Med. 1, 621–622.
212. Champagne, P., Ogg, G. S., King, A. S., Knabenhans, C., Ellefsen, K., Nobile, M., Appay, V., Rizzardi, G. P., Fleury, S., Lipp, M., Forster, R., Rowland-Jones, S., Sekaly, R. P., McMichael, A. J., Pantaleo, G. (2001) Skewed maturation of memory HIV-specific CD8 T lymphocytes. Nature 410, 106–111.
213. Soumelis, V., Scott, I., Gheyas, F., Bouhour, D., Cozon, G., Cotte, L., Huang, L., Levy, J. A., Liu, Y-J. (2001) Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. Blood 98, 906–912.
214. Murray, H. W., Hillman, J. K., Rubin, B. Y., Kelly, C. D., Jacobs, J. L., Tyler, L. W., Donelly, D. M., Carriero, S. M., Godbold, J. H., Roberts, R. B. (1985) Patients at risk for AIDS-related opportunistic infections. Clinical manifestations and impaired gamma interferon production. N. Engl. J. Med. 313, 1504–1510.
215. Lane, H. C., Depper, J. M., Greene, W. C., Whalen, G., Waldmann, T. A., Fauci, A. S. (1985) Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. N. Engl. J. Med. 313, 79–84.
216. Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M., Markowitz, M. (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373, 123–126.
217. Meyaard, L., Otto, S. A., Mijnster, M. J., Keet, R. P., Miedema, F. (1992) Programmed death of T cells in HIV-1 infection. Science 257, 217–219.
218. Herbein, G., Mahlknecht, U., Batliwalla, F., Gregersen, P., Pappas, T., Butler, J., O'Brien, W. A., Verdin, E. (1998) Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. Science 395, 189–194.
219. Rowland-Jones, S., McMichael, A. J. (1993) Cytotoxic T lymphocytes in HIV infection. Semin. Virol. 4, 83–94.
220. Borrow, P., Lewicki, H., Hahn, B. H., Shaw, G. M., Oldstone, M. B. (1994) Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. 68, 6103–6110.
221. Carmichael, A., Jin, X., Sissons, P., Borysiewicz, L. (1993) Quantitative analysis of the human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) response at different stages of HIV-1 infection: differential CTL responses to HIV-1 and Epstein-Barr virus in late disease. J. Exp. Med. 177, 249–256.
222. Chun, T. W., Justement, J. S., Moir, S., Hallahan, C. W., Ehler, L. A., McLaughlin, M., Dybul, M., Mican, J. M., Fauci, A. S. (2001) Suppression of HIV replication in the resting CD4+ T cell reservoir by autologous CD8+ T cells: implications for the development of therapeutic strategies. Proc. Natl. Acad. Sci. USA 98, 253–258.
223. Napolitano, L. A., Grant, R. M., Deeks, S. G., Schmidt, D., De Rosa, S. C., Herzenberg, L. A., Herndier, B. G., Andersson, J., McCune, J. M. (2001) Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Nat. Med. 7, 73–79.
224. Rosenberg, E. S., Billingsley, J. M., Caliendo, A. M., Boswell, S. L., Sax, P. E., Kalams, S. A., Walker, B. D. (1997) Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 278, 1447–1450.
225. Huber, D., Benyoucef, S., Chehadeh, W., Chieux, V., De La Tribonniere, X., Mouton, Y., Bocket, L., Wattre, P. (1998) Production of interleukin-4, interferon (IFN)-gamma and IFN-alpha in human immunodeficiency virus-1 infection: an imbalance of type 1 and type 2 cytokines may reduce the synthesis of IFN-alpha. Scand. J. Immunol. 48, 436–442.
226. Walker, C. M., Moody, D. J., Stites, D. P., Levy, J. A. (1986) CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 234, 1563–1566.
227. Levy, J. A., Mackewicz, C. E., Barker, E. (1996) Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8+ T cells. Immunol. Today 17, 217–224.
228. Yang, O. O., Walker, B. D. (1997) CD8+ cells in human immunodeficiency virus type 1 pathogenesis: cytolytic and noncytolytic inhibition of viral replication. Adv. Immunol. 66, 273–311.
229. Tomaras, G. D., Lacey, S. F., McDanal, C. B., Ferrari, G., Weinhold, K. J., Greenberg, M. L. (2000) CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. Proc. Natl. Acad. Sci. USA 97, 3503–3508.
230. Walker, B. D., Plata, F. (1990) Cytotoxic T lymphocytes against HIV. AIDS 4, 177–184.
231. D'Souza, M. P., Cairns, J. S., Plaeger, S. F. (2000) Current evidence and future directions for targeting HIV entry: therapeutic and prophylactic strategies. J. Am. Med. Assoc. 284, 215–222.
232. Rossi, D., Zlotnik, A. (2000) The biology of chemokines and their receptors. Annu. Rev. Immunol. 18, 217–242.
233. Wiviott, L. D., Walker, C. M., Levy, J. A. (1990) CD8+ lymphocytes suppress HIV production by autologous CD4+ cells without eliminating the infected cells from culture. Cell. Immunol. 128, 628–634.
234. Walker, C. M., Erickson, A. L., Hsueh, F. C., Levy, J. A. (1991) Inhibition of human immunodeficiency virus replication in acutely infected CD4+ cells by CD8+ cells involves a noncytotoxic mechanism. J. Virol. 65, 5921–5927.
235. Mackewicz, C. E., Yang, L. C., Lifson, J. D., Levy, J. A. (1994) Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection. Lancet 344, 1671–1673.
236. Wang, Y., Tao, L., Mitchell, E., Bruvery, C., Berlingieri, P., Armstrong, P., Vaughan, R., Underwood, J., Lehner, T. (1999) Allo-immunization elicits $CD8^+$ T cell-derived chemokines, HIV suppressor factors and resistance to HIV infection in women. Nat. Med. 5, 1004–1009.
237. Jansson, M., Backstrom, E., Bjorndal, A., Holmberg, V., Rossi, P., Fenyo, E. M., Popovic, M., Albert, J., Wigzell, H. (1999) Coreceptor usage and RANTES sensitivity of non-syncytium-inducing HIV-1 isolates obtained from patients with AIDS. J. Hum. Virol. 2, 325–338.
238. Addo, M. M., Altfeld, M., Rosenberg, E. S., Eldridge, R. L., Phillips, M. N., Habeeb, K., Khatri, A., Brander, C., Robbins, G. K., Mazzara, G. P., Goulder, P. J., Walker, B. D. (2001) The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals. Proc. Natl. Acad. Sci. USA 98, 1781–1786.
239. Klein, M. R., van Baalen, C. A., Holwerda, A. M., Kerkhof Garde, S. R., Bende, R. J., Keet, I. P., Eeftinck-Schattenkerk, J. K., Osterhaus, A. D., Schuitemaker, H., Miedema, F. (1995) Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. J. Exp. Med. 181, 1365–1372.
240. Michael, A. J., Ogg, G., Wilson, J., Callan, M., Hambleton, S., Appay, V., Kelleher, T., Rowland-Jones, S. (2000) Memory CD8+ T cells in HIV infection. Philos. Trans. R. Soc. Lond. 355, 363–367.
241. Berger, E. A., Murphy, P. M., Farber, J. M. (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. 17, 657–700.
242. Flamand, L., Crowley, R. W., Lusso, P., Colombini-Hatch, S., Margolis, D. M., Gallo, R. C. (1998) Activation of CD8+ T lymphocytes through the T cell receptor turns on CD4 gene expression: implications for HIV pathogenesis. Proc. Natl. Acad. Sci. USA 95, 3111–3116.
243. Cullen, B. R. (2001) A new entry route for HIV. Nat. Med. 7, 20–21.
244. Saha, K., Zhang, J., Gupta, A., Dave, R., Yimen, M., Zerhouni, B. (2001) Isolation of primary HIV-1 that target CD8+ T lymphocytes using CD8 as a receptor. Nat. Med. 7, 65–72.
245. Roy, S., Katze, M. G., Parkin, N. T., Edery, I., Hovanessian, A. G., Sonenberg, N. (1990) Control of the interferon-induced 68-kilodalton protein kinase by the HIV-1 tat gene product. Science 247, 1216–1219.
246. McMillan, N. A., Chun, R. F., Siderovski, D. P., Galabru, J., Toone, W. M., Samuel, C. E., Mak, T. W., Hovanessian, A. G., Jeang, K. T., Williams, B. R. (1995) HIV-1 Tat directly interacts with the interferon-induced, double-stranded RNA-dependent kinase, PKR. Virology 213, 413–424.
247. Collins, K. L., Chen, B. K., Kalams, S. A., Walker, B. D., Baltimore, D. (1998) HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature 391, 397–401.
248. Aiken, C., Konner, J., Landau, N. R., Lenburg, M. E., Trono, D. (1994) Nef induces CD4 endocytosis: requirement for a critical dileucine motif in the membrane-proximal CD4 cytoplasmic domain. Cell 76, 853–864.
249. Ho, D. D., Hartshorn, K. L., Rota, T. R., Andrews, C. A., Kaplan, J. C., Schooley, R. T., Hirsch, M. S. (1985) Recombinant human interferon alfa-A suppresses HTLV-III replication in vitro. Lancet 1, 602–604.
250. Fernie, B. F., Poli, G., Fauci, A. S. (1991) Alpha interferon suppresses virion but not soluble human immunodeficiency virus antigen production in chronically infected T-lymphocytic cells. J. Virol. 65, 3968–3971.
251. Shirazi, Y., Pitha, P. M. (1992) Alpha interferon inhibits early stages of the human immunodeficiency virus type 1 replication cycle. J. Virol. 66, 1321–1328.
252. Meylan, P. R., Guatelli, J. C., Munis, J. R., Richman, D. D., Kornbluth, R. S. (1993) Mechanisms for the inhibition of HIV replication by interferons-alpha, -beta, and -gamma in primary human macrophages. Virology 193, 138–148.
253. Coccia, E. M., Krust, B., Hovanessian, A. G. (1994) Specific inhibition of viral protein synthesis in HIV-infected cells in response to interferon treatment. J. Biol. Chem. 269, 23087–23094.
254. Baca-Regen, L., Heinzinger, N., Stevenson, M., Gendelman, H. E. (1994) Alpha interferon-induced antiretroviral activities: restriction of viral nucleic acid synthesis and progeny virion production in human immunodeficiency virus type 1-infected monocytes. J. Virol. 68, 7559–7565.
255. Kinzl, P., Otani, T., Benz, R., Minowada, J. (2001) Interferon-alpha and -gamma differentially reduce rapid immature T-cell death by contact with HIV-1 carrier cell clones in vitro. Microbiol. Immunol. 41, 709–716.